GROWTH REGULATION OF CANCER-II

Organizers: Marc Lippman and Robert Dickson
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New Oncogenes in Human Malignancy

E 001 ANALYSIS OF DIFFERENTIAL EXPRESSION OF FUNCTION OF MYC-FAMILY AND RELATED GENES IN NORMAL AND TRANSFORMED CELLS. Frederick Alt, Renate Dildrop, Robert Collum, Tarik Moroy, Kathryn Zimmerman, Averil Ma, Jean Charron, Steven Goff, and Ron DePinho. Howard Hughes Medical Institue and Departements of Biochemistry and Microbiology, College of Physcian and Surgeons of Columbia University, New York, N.Y. 10032. Expression of the N-, L-, and c-myc genes is differentially regulated during normal murine development. For example, both N- and c-myc are expressed in precursor B and T lymphocytes but only c-myc is expressed after the cells differentiate into mature lymphoctyes. To study the differential control and functions of myc-family genes, we have generated a variety of transgenic mouse lines that contain introduced N- or L-myc genes expressed under the influence of their own regulatory elements or specifically-targeted and deregulated within the lymphoid lineage. In addition, we have disrupted one copy of the endogenous N-myc gene in embryonic stem cells by homologous recombination; these cells have been used for blastocyst injections and resulting chimaeric mice are being anlyzed for germline transmission. We will discuss results of these studies in the context of differential myc-gene regulation and activities in normal and transformed cells. We will also desribe a novel human gene that, in preliminary analyses, has myc-like activity in the rat embryo fibroblast co-transformation assay and has a highly restricted developmental- and tumor-specific expression pattern.

E 002 ONCOGENES INTERFERE WITH MORPHOLOGICAL AND FUNCTIONAL DIFFERENTIATION OF MAMMARY EPITHELIAL CELLS.

Bernd Groner, Roland Ball, Wolfgang Doppler, Anne Catherine Andres and Nancy E. Hynes. Friedrich Miescher Institut, P.O. Box 2543, CH-4002 Basel, Switzerland.

We are investigating the development, differentiation and transformation of the cells of the mammary epithelium. In vitro and in vivo studies have been carried out to gain insights in the process of terminal differentiation and lactogenic hormone responsiveness, the effects of oncogenes on morphological and functional mammary gland differentiation in the mouse and the involvement of oncogenes in primary human breast cancer. (1) A clonal mouse mammary epithelial cell line (HC11) has been derived which grows continuously in culture and which can undergo terminal differentiation under appropriate culture conditions. Extended periods of confluence and treatment with glucococrticoid hormones cause the responsiveness of the HC11 cells to the lactogenic action of prolactin. Sequence elements in the β casein gene promoter, located in the 5' upstream region between positions -285 and -170, have been identified by gene transfer experiments to contain cis acting sequences mediating the synergistic effect of prolactin and glucocorticoid hormones. An increase in the expression of the mouse c-erbB-2 gene accompanies the terminal differentiation process. (2) Targeted expression of oncogenes has been used to study the effects of Ha-ras and c-myc on the mammary epithelium. Recombination of the oncogene coding regions with a milk protein (whey acidic protein) gene specific promoter resulted in the mammary gland specific and lactogenic hormone dependent expression of the oncoproteins. The Wap-myc transgene is highly tumorgenic. Tumorgenicity is preceded by a interference with the process of terminal differentiation and postlactational tissue regression. Tumor cells grow independently of the hormone status and induce extracellular matrix protein (tenascin) expression in surrounding mesenchyme. (3) Primary human tumor samples from stage 1 breast cancer patients have been evaluated by immunohistochemical means for the expression of the c-erbB-2 gene, the EGF-receptor gene and ras genes. The extent of gene expression has been correlated with course of disease. Although c-erbB-2 expression alone did not allow predictions concerning relapse or survival of patients, combinations with other prognostic factors proved to be informative. The best overall survival in the group of node negative patients was observed when the primary tumors were estrogen receptor positive and c-erbB-2 negative. The combination of several prognostic variables might gain importance in the diagnosis of breast cancer.

Tyrosine Kinase-Related Oncogenes

E 003 **CD4 SIGNAL TRANSDUCTION IN T LYMPHOCYTES INVOLVES THE CELLULAR TYROSINE PROTEIN KINASE p56^{lck}.** Andre Veillette[1], Michael A. Bookman[2], Lawrence E. Samelson[3], and Joseph B. Bolen[1]. Laboratory of Tumor Virus Biology[1] and Medicine Branch[2], National Cancer Institute, Cell Biology and Metabolism Branch[3], National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.

Expression of the CD4 glycoprotein on the surface of mature T-lymphocytes phenotypically correlates with the ability of a T-cell to recognize class II major histocompatibility complex determinants as restriction elements on antigen presenting cells. Accumulating evidence suggests that in addition to potentially stabilizing physical interactions between cells, CD4 is also capable of generating a signal which enhances T-cell receptor dependent signals during the process of T lymphocyte activation. We have recently determined that p56^{lck} (a member of the src-family of tyrosine protein kinase proto-oncogenes) forms a non-covalent complex with CD4 molecules in murine and human T lymphocytes. To determine whether CD4 signalling events involve p56^{lck}, the effects of antibody mediated CD4 cross-linking were evaluated and compared with cross-linking of other T-cell surface antigens. The results of these experiments demonstrate that CD4 cross-linking specifically induces rapid stimulation of p56^{lck} functions through altering the specific activity of CD4 associated p56^{lck}. These results indicate that CD4 signalling pathways involve modulation of p56^{lck} activity through mechanisms reminiscent of the tyrosine kinase activation events following ligand binding and cross-linking of peptide hormone receptors.

E 004 PROPERTIES OF THE MET ONCOGENE AND PROTO-ONCOGENE, G.F. Vande Woude[1], M. Bodescot[1], D. Blair[2], M.I. Gonzatti[1], T. Kmiecik[1], M. Park[3], A.Iyer[4], and S. Showalter[5], [1]BRI-Basic Research Program and [2]Laboratory of Molecular Oncology, NCI-Frederick Cancer Research Facility, Frederick, MD 21701; [3]Ludwig Institute, Montreal, Canada; [4]Rush Presbyterian St. Luke's Medical Center, Chicago, IL 60612; and [5]Program Resources, Inc.

The met proto-oncogene is a member of the tyrosine-kinase growth-factor receptor gene family and proteins encoded by the human proto-oncogene (p170^{met} and p140^{met}) and the tpr-met oncogene (p65$^{tpr\text{-}met}$) have been identified (Gonzatti et al., PNAS 85:21-25, 1988). We have characterized the synthesis and processing of the proto-oncogene product. p170^{met} is the major product detected in a 30-minute pulse, but rapidly disappears during the chase ($t_{\frac{1}{2}}$ = 2.5 h). p140^{met} is first detected after a 60-minute chase ($t_{\frac{1}{2}}$ = 12 h) and is the mature cell surface product. An antibody directed against N-terminal amino acids 212-231 recognizes p170^{met}, but fails to recognize p140^{met} suggesting that processing requires a novel N-terminal proteolytic cleavage. In the presence of tunicamycin, only a 150-kd product is detected. Likewise, met mRNA transcribed from an SP6 met cDNA vector and translated in vitro yields a 150-kd protein. In the presence of canine pancreatic microsomal membranes which can mediate N-linked glycosylation, the product is the same size as in vivo synthesized p170^{met}. These results suggest that p150 is glycosylated to yield p170^{met} which is subsequently cleaved to yield p140^{met}. Under nonreducing conditions, a 200 kd met proto-oncogene product is detected suggesting that the moiety cleaved from the N terminus may remain associated through sulfhydryl linkage to the p140^{met}.

To study the met proteins and their transforming potential, cDNAs derived from the tpr-met oncogene and the human and mouse met proto-oncogenes were placed under the transcriptional control of the Moloney MSV-LTR and introduced into mouse NIH/3T3 cells by DNA transfection. We find that the tpr-met oncogene cDNA efficiently transforms NIH/3T3 cells. There is no difference in the transforming activity when the kinase domain of the oncogene is replaced with that of the proto-oncogene. We have found that the mouse met proto-oncogene cDNA transforms NIH/3T3 cells, while the human met proto-oncogene cDNA does not. Chimeras between the mouse and human cDNAs can serve to identify the domain(s) which is responsible for the transforming activity.

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Growth Factors

E 005 EXPRESSION OF THE c-erbB-2 PROTEIN IN HUMAN BREAST CANCER AND ITS ROLE AS AN INDICATOR OF DESEASE PROGRESSION, William J. Gullick, ICRF Oncology Group, MRC Cyclotron Building, Hammersmith Hospital, Du Cane Road, London W12 OHS, U.K.

We have examined the structure and function of the c-erbB-2 protein in normal and malignant tissues. Elevated expression of normal rat neu/c-erbB-2 protein in NIH 3T3 cells appears to reduce the gap-junctional permiability of the cells as observed by microinjection of an indicator dye. A chimera consisting of the external domain of the EGF receptor and the cytoplasmic domain of neu (constructed by Dr. K. Alitalo and colleagues, Helsinki) was shown to respond to the addition of EGF by increased autophosphorylation.

The level of expression of the c-erbB-2 protein has been examined by immunohistological staining of a variety of normal human foetal and adult tissues. Moderate levels of expression were found on brian, kidney, liver, striated muscle and secretory epithelia. Relatively elevated levels of c-erbB-2 protein expression associated with gene amplification were observed in human breast and stomach cancer biopsies. The presence of elevated levels of c-erbB-2 protein found in approximately 15% of human breast adenocarcinomas appears to define a group of patients with a relatively short desease free interval and survival. Elevated expression of the EGF receptor was an independent predictor of poor prognosis. These results suggest that overexpression of growth factor receptors may play a role in the developement of human breast cancer and may provide an indicator of desease progression.

Growth Factor Receptors and Mutants

E 006 **GENE EXPRESSION IN PROLIFERATION AND TRANSFORMATION.** Harvey R. Herschman. Department of Biological Chemistry and Laboratory of Biomedical and Environmental Sciences. UCLA School of Medicine, Los Angeles, CA 90024

We have isolated mutants of 3T3 cells unable to mount a proliferative response to specific mitogens. Two epidermal growth factor (EGF) nonproliferative mutants, 3T3-NR6 and 3T3-TNR2, have no immunologically cross-reactive EGF receptor mRNA; they are "shut down" at the level of EGF receptor gene expression. We have also isolated two Swiss 3T3 variants, 3T3-TNR2 and 3T3-TNR9, unable to respond proliferatively to tetradecanoyl phorbol acetate (TPA). The Balb/c 3T3 mutant E12a (isolated by Tom O'Brien and his colleagues), shares a similar phenotype. All three TPA nonproliferative variants retain TPA-activatible protein kinase C. We have recently isolated a family of primary response genes induced by TPA; we term these genes TPA Inducible Sequences, or TIS genes. One TIS gene, TIS28, is c-fos; a second TIS gene, TIS8, is Egr-1. Sequence data suggests that the other TIS genes are not related to sequences entered in the common data bases. All the TIS genes assayed to date are expressed in the three TPA nonproliferative variants; the lesion(s) in the TPA nonproliferative variants must occur either distal to the expression of TIS genes, or in a distinct TPA-induced pathway.

The TIS genes are induced by other 3T3 mitogens, such as EGF and fibroblast growth factor (FGF). One might suspect, since these genes were first described as a consequence of TPA induction, that EGF and FGF induction of the TIS genes might be exclusively or primarily mediated by a protein kinase C pathway. However, down regulation of protein kinase C by TPA reduces EGF and FGF induction of the TIS genes only slightly. Independent mitogen-stimulated pathways to TIS gene expression must exist.

The TIS genes are induced in response to differentiation signals as well as mitogenic signals. Nerve growth factor induces expression of a subgroup of the TIS genes in the PC12 pheochromocytoma cell line. Granulocyte-macrophage stimulating factor (GM-CSF) induces a distinct subset of the TIS genes in GM-CSF proliferation dependent myeloid cells and in post-mitotic neutrophils. Cell-type specific responses to common ligands may be mediated by developmental restriction of the constellation of "third messenger" genes represented by the TIS genes.

E 007 MUTATIONAL ANALYSIS OF THE EGF-RECEPTOR, Schlessinger J., Rorer Biotechnology, Inc., King of Prussia PA, 19406 and Ullrich A., Genentech, South San Francisco, CA 94080. The membrane receptor of epidermal growth factor (EGF-receptor) is composed of a large extracellular ligand binding domain, a single transmembrane region and a cytoplasmic domain containing protein tyrosine kinase activity. We have formulated an allosteric oligomerization model for activation of the catalytic properties of the kinase domain by subunit interaction between neighboring cytoplasmic domains (1). In vitro site directed mutagenesis was used to generate various EGF-receptor mutants. NIH-3T3 cells lacking endogenous EGF-receptors were transfected with different constructs and shown to express the following mutant EGF receptors: 1) A kinase negative point mutant of EGF receptor, 2) mutant receptors with altered autophosphorylation sites, 3) mutant receptor with altered transmembrane regions, 5) chimeric interspecies chicken/human EGF receptor mutants for the identification of ligand binding domain, 6) chimeric neu/EGF receptor mutants. Using this approach we have shown that the kinase activity of EGF receptor is essential for signal transduction (1,2) while autophosphorylation is not crucial for receptor signalling (3). Moreover, the kinase activity is also essential for normal receptor trafficking; instead of entering a degradative pathway, the kinase negative mutant EGF-receptor undergoes continuous recycling to the cell surface for reutilization. The precise composition of the transmembrane domain is not essential for receptor activity (4), further supporting the oligomerization model for receptor activation (1). Finally, we have also identified domain III flanked by the two cysteine domains of the extracellular domain as a major binding domain (5,6).

1. Schlessinger, J. (1988) Biochemistry 27, 3119-3123.
2. Honegger et al (1987) Cell 51, 199-209.
3. Honegger et al (1988) EMBO J. 7, 3045-3052.
4. Kashles et al (1988) Proc. Natl. Acad. Sci. USA (1988) in press.
5. Lax et al (1988) Mol. Cell. Biol. 8, 1831-1834.
6. Lax et al (1988) EMBO J. submitted.

Integrated Systems

E 008 Molecular Genetic Studies on Mammalian GRP

James F. Battey*, Anne Marie Lebacq-Verheyden*, R. Thomas Zoeller**, and James Way*. *Laboratory of Neurochemistry, NINCDS, NIH, Bethesda, MD 20892 and **Department of Anatomy, University of Missouri School of Medicine, Columbia, MO 65212.

Gastrin releasing peptide (GRP) is the mammalian homolog to the amphibian peptide bombesin. It functions as a digestive hormone, a neuropeptide, and a growth factor for Swiss 3T3 murine embryonal fibreblasts, pulmonary epithelial cells and some small cell lung carcinoma cells. Expression of the human GRP gene is restricted to a limited subset of neural and neuroendocrine cells. Gene regulatory studies show that the level of steady state mRNA is regulated at the level of primary transcription, and that a cluster of DNase hypersensitive sites in the promoter region is invariably associated with transcription. Post translational processing of human proGRP is an essential prerequisite to the production of a GRP peptide capable of binding high affinity cell surface receptors that mediate all known biological effects. Processing of proGRP differs in a number of cell types examined and provides another level of regulation on the levels of GRP produced. In situ hybridization studies on rat brain show that GRP gene expression is detectable in a limited subset of neurons in specific anatomical regions. These regions correlate well with the previously determined location of GRP receptors, suggesting that GRP is acting locally as a neurotransmitter in the brain.

FGF and Related Growth Factors

E 009 EXPRESSION AND PROPERTIES OF THE GROWTH FACTOR ENCODED IN THE K-fgf ONCOGENE. Claudio Basilico, Anna Maria Curatola, Karen Newman, Daniela Talarico, Alka Mansukhani, Michael Ittmann, Anna Velcich and Pasquale Delli-Bovi. Department of Pathology and Kaplan Cancer Center, New York University School of Medicine, New York, NY 10016.

The K-fgf oncogene, isolated by transfection of Kaposi's Sarcoma DNA, encodes a novel growth factor with significant homology to basic and acidic FGFs. To study the properties of this growth factor and the mechanism by which the K-fgf oncogene transforms cells, we have studied the production and processing of the K-FGF protein in COS and CHO cells containing a high copy number of K-fgf cDNA. Unlike basic and acidic FGFs, the K-FGF protein is cleaved after a signal peptide, glycosylated and efficiently secreted as a mature protein of 176 amino acids. Cells transformed by the K-fgf oncogene secrete the growth factor and grow in serum-free medium, consistent with an autocrine mechanism of growth. We have investigated the mechanism of activation of the K-fgf oncogene by cloning both the human and mouse corresponding protooncogenes and determining their transforming ability and pattern of expression. The cloned K-fgf protooncogene can transform cells in culture, and encodes a protein identical to K-FGF. These results and the fact that K-fgf expression is virtually undetectable in a variety of "normal" cell lines and tissues indicate that the activation of this oncogene is not due to mutations in the coding sequences, but rather to unregulated expression. The K-fgf protooncogene is expressed at significant levels in undifferentiated mouse and human teratocarcinoma cell lines, and induction of differentiation shuts-off expression, indicating that K-FGF may play a role in early development. Comparison of the biological activity of K-FGF with that of bFGF so far indicates that all cells which respond to bFGF are also sensitive to K-FGF, and the same is true for cells which do not respond. Together with other experiments that will be discussed these data suggest that, although the pattern of expression of their respective genes is very different, bFGF and K-FGF act through binding to the same cellular receptor.

E 010 THE INT-2 GENE; A PROTEIN IMPLICATED IN NORMAL FETAL DEVELOPMENT AND VIRALLY INDUCED MAMMARY TUMORIGENESIS, C. Dickson, M. Dixon, R. Deed, R. Smith, S. Brookes, P. Acland and G. Peters, Imperial Cancer Research Fund, London, U. K.

The int-2 gene encodes a 27 kilodalton product that shows significant homology to the fibroblast growth factor family. The gene was originally discovered as a common site for provirus integration in carcinomas induced by mouse mammary tumor virus (MMTV). Proviruses are found on either side of int-2 in a configuration in which viral transcription is directed away from the gene. Integration at this locus directly correlates with its transcriptional activation, presumably by cis acting viral enhancers. The gene is not detectably expressed in normal mammary gland nor in most adult tissues surveyed. However, during normal mouse development, int-2 appears to function at several different stages of embryogenesis, seemingly under strict spacial and temporal control. To gain some insight into the control mechanisms involved, we have examined the expression of the int-2 gene in embryonal carcinoma cell lines induced to differentiate with retinoic acid and dibutyryl cyclic AMP, as well as in MMTV-induced mammary tumors. At least six different classes of int-2 RNA can be distinguished, initiating at three distinct promoters and terminating at either of two alternative polyadenylation sites. Despite the structural complexity, these RNA transcripts appear to encode the same protein. To begin to characterize this product we have prepared a series of polyclonal and monoclonal antibodies directed against synthetic peptides, and to parts of the int-2 protein expressed in bacteria. Several of these sera are able to detect candidate int-2 proteins by Western blotting and immunocytochemical procedures. Multiple forms of int-2 protein can be detected suggesting post-translational modification. Immunoperoxidase staining of over-expressing COS cells indicates localization within the endoplasmic reticulum and golgi. These observations are consistent with the idea that int-2 may be an extracellular growth modulator that normally functions during embryonic development and whose function is subverted in virally induced mammary tumors.

E 011 THE int ONCOGENES IN MAMMARY TUMORIGENESIS AND IN NORMAL DEVELOPMENT, R. Nusse, H. Roelink, F. Rijsewijk, M. van de Heuvel and E. Wagenaar, Division of Molecular Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

The Mouse Mammary Tumor Virus causes mammary tumors by proviral insertion near host cell oncogenes, collectively called int. The int-1 gene encodes a cysteine-rich protein with all the characteristics of a secretory factor. The gene is expressed during neurogenesis of the mouse and in adult testis. The role of int-1 in normal development was underscored by its homology to the Drosophila segment polarity gene wingless. Wingless is involved in various forms of pattern formation, in segments and in imaginal discs. We study interactions of wingless and other segmentation genes in Drosophila, by gene transfer experiments and by localization of the wingless product. Antibodies have been raised to the wingless protein, which stain, in a 14 stripe pattern, the surface of cells adjacent to the parasegment boundary. Wingless protein expression is adjacent to cells with engrailed expression. A transgenic Drosophila strain carrying an Hsp70-wingless construct shows indiscriminate expression of the wingless protein. The pattern abnormality in transgenic embryos indicates that the wingless protein is able to cause differentiation abnormalities wherever it is expressed (collaboration with Dr. P. Lawrence, MRC, Cambridge, U.K.).

The int-4 gene, cloned recently in our lab, is expressed in mouse embryogenesis at specific sites and time intervals. The gene is highly conserved in evolution, as concluded from heteroduplex analysis between the mouse and the human int-4 homologues.

TGF; New Oncogenes

E 100 EFFECTS OF TGF-BETA1 AND MATRIX PROTEINS ON ANCHORAGE-INDEPENDENT GROWTH OF NRK FIBROBLASTS. Richard K. Assoian, Lori A. Boardman, and Sophia Drosinos, Department of Biochemistry and Molecular Biophysics, and the Center for Reproductive Sciences, College of Physicians and Surgeons, Columbia University, New York, NY 10032

We have developed a preparative suspension culture system for examining anchorage-independent growth of NRK fibroblasts. In this system, growth factors and cells are suspended above a soft agar bottom layer. Cell division occurs in response to the synergistic interaction of TGF-beta1 and EGF in a manner similar to that observed with traditional analytical soft agar assays. However, since our system permits recovery of viable cells, mitosis and colony formation can be quantitated objectively by incorporation of ^{3}H-thymidine into newly synthesized DNA. We have used this system to compare the effects of TGF-beta1, collagen I and fibronectin on stimulation of anchorage-independent growth. Quantitative reconstitution experiments show that collagen I and fibronectin, used at concentrations optimal for attachment of NRK cells in monolayer, have no ability to stimulate mitosis of serum/EGF-treated NRK cells in suspension. Moreover, NRK cells phenotypically transformed by TGF-beta1 can grow as single cells, and not as colonies, as long as their movement within the culture system is not precluded by agar or methylcellulose. We conclude that the well-established effects of TGF-beta1 on stimulation of collagen I and fibronection synthesis are not sufficient to induce division of suspended NRK cells, and that phenotypic transformation by TGF-beta1 is a true reflection of anchorage-independent growth. Supported by NIH grant HL38884.

E 101 ACTIVATION OF A NOVEL HUMAN ONCOGENE REVEALS A LOCUS UBIQUITIOUSLY EXPRESSED IN CELLS OF HEMATOPOIETIC LINEAGE, Shulamit Katzav*, Dionisio Martin-Zanca* and Mariano Barbacid*†. Basic Research Program, Frederick Cancer Research Facility, Frederick, MD 21701*, and The Squibb Institute for Medical Resarch, Princeton, NJ 08543†.

We have isolated a novel human oncogene activated during the course of gene transfer assays. This oncogene acquired its transforming properties by a fortuitous rearrangement that placed the coding sequences of a novel human gene under the regulatory control of the SV40 early promoter present in the co-transfected pSV2neo DNA, used as a selectable marker. This human gene, designated vav, codes for a 725 amino acid long polypeptide that exhibits several motifs characteristic of transcriptional factors. These motifs include a highly acidic 45 amino acid long domain and two zinc finger-like regions separated by a putative proline reach (PPSP) hinge region. In addition, the vav gene product contains two nuclear localization signals and a potential phosphorylation site for protein kinase A. Ectopic expression of this protein in NIH3T3 cells induces their malignant transformation, suggesting that expression of the vav gene must be either tightly controlled or be restricted to cells of specific developmental lineages. We have observed vav gene expression in over 40 hematopoietic cell lines of human and mouse origin including cells representative of the major developmental lineages, lymphoid, myeloid and erythroid. We have also identified vav gene transcripts in normal B and T lymphocytes, macrophages and platelets. In contrast, we have not observed detectable vav gene expression in cells of any other developmental lineage. These properties suggest that the vav gene product may participate in the transcriptional machinery that controls the proliferative stage of the hematopoietic system.

E 102 CO-AMPLIFICATION OF BCL-1 AND INT-2 GENES IN HEAD AND NECK SQUAMOUS CARCINOMAS, James Berenson, Jie Yang and Robert Mickel, Departments of Medicine and Surgery, UCLA School of Medicine and Wadsworth VA Medical Center, Los Angeles, CA 90024

The putative oncogenes bcl-1 and int-2 are located on the long arm of chromosome 11 at band q13. Int-2 is one of the integration sites for the murine mammary tumor virus, and the human genome contains homologous DNA. Bcl-1 is located at the breakpoint of the t(11;14) (q13; q32) translocation which is found in some B cell malignancies. In this investigation, the arrangement, copy number and expression of the bcl-1 and int-2 genes were determined in six head and neck squamous cell carcinoma (HNSCC) cell lines and in the tumors and peripheral blood of 17 patients with HNSCC. Moreover, karyotypic analysis was determined on the six HNSCC cell lines which included CAL-27 and University of Michigan (UMSCC) 1, 8, 16, 19, 22A. DNA was isolated from granulocytes (germline), fresh tumor and cell lines, and digested with restriction endonucleases. Total RNA was isolated from fresh tumor and cell lines. DNA was subjected to Southern blot analysis with bcl-1, int-2, and human beta TCR (single copy control) gene probes. Northern blot analysis was performed using the bcl-1, int-2 and actin (control) gene probes. We demonstrated that the bcl-1 gene was amplified three to 10-fold in the tumors of six of seventeen patients. Int-2 was also amplified to the same degree in the six tumors with bcl-1 amplification. These two genes were also co-amplified in two of the six HNSCC cell lines (UMSCC 8 and 22A). There was no rearrangement of either gene in any tumor samples or cell lines. Karyotypic analysis revealed consistent abnormalities of chromosome 11 in only UMSCC 8 and 22A. Northern blot analysis of the six cell lines and fresh tumors did not demonstrate bcl-1 or int-2 expression.

E 103 THE TGF-α PRECURSORS INDUCE PHOSPHORYLATION OF EGF-RECEPTORS, Rainer Brachmann and Rik Derynck, Department of Developmental Biology, Genentech, Inc., South San Francisco, CA 94080 Immunofluorescence studies with a monoclonal anti-TGF-α antibody could demonstrate membrane associated TGF-α on the surface of TGF-α overexpressing CHO cells, as well as on transformed cell lines HT1080 and 7860. To determine a possible biological function of transmembranous TGF-α precursors, the two processing sites at the C-terminus of TGF-α were changed by site-directed mutagenesis of the cDNA and the modified coding sequence was overexpressed in CHO cells. The TGF-α precursors were purified by immunoaffinity column and determination of the NH_2-terminal sequence established the presence of two forms of the precursor.

The preparation of two TGF-α precursors stimulated the phosphorylation of EGF-receptors in A431 cells. Amino acid analysis data suggests, that the solubilized TGF-α precursors are 50- to 100- fold less active than mature TGF-α. These data imply, that the TGF-α precursors can exert an activity in cell-to-cell contact. This may reflect an early evolutionary form of cell communication.

E 104 GENE EXPRESSION OF TRANSFORMING GROWTH FACTOR-α IN HUMAN RENAL CARCINOMAS: CORRELATION WITH TUMOR PROGRESSION,Jutta Bovens, Gerhard Jakse, Rik Derynck and Petro E. Petrides, Laboratory of Molecular Oncology, Department of Medicine III,Munich University School of Medicine, Marchioninistrasse 15, D-8000 Munich 70
Investigations of the last years revealed, that growth factors play an important role in tumor formation and progression. Two major classes of transforming growth factors have been identified, purified and cloned, TGF-α and TGF-β. Since the cDNA for TGF-α was originally isolated from a renal carcinoma cell line, we have decided to study human renal carcinomas for the expression of mRNA coding for TGF-α using the Northern blotting technique. Tumor tissue was obtained by nephrectomy and frozen in liquid nitrogen until use. All renal carcinomas were found to express a specific 4.8 kb TGF-α mRNA. For comparison, adjacent normal tissue from the same kidney was tested. Although the gene was expressed in some normal renal tissue, the degree of expression was significantly higher in transformed regions of the kidney. Our results suggest that quantitative differences in TGF-α expression correspond with tumor progression and differentiation of the tumor. Thus, high expression of TGF-α indicates poorly or undifferentiated carcinomas. Whether these results represent a special property of renal carcinomas or can be generalized, is part of current investigations. Supported in part by Boehringer Ingelheim Fonds, Stiftung für medizinische Grundlagenforschung.

E 105 SITE-DIRECTED MUTAGENESIS OF CYSTEINE RESIDUES LOCATED IN THE AMINO-TERMINAL PRECURSOR REGION OF TGF-β1: EXPRESSION AND CHARACTERIZATION OF MUTANT PROTEINS, Amy M. Brunner and A.F. Purchio, Oncogen, 3005 First Avenue, Seattle, WA 98112. Both precursor and mature forms of TGF-β1 are secreted by CHO cells transfected with cDNA constructs encoding Simian TGF-β1 (Gentry et al., 1987, Mol. Cell. Biol. 7, 3418). On non-reducing SDS-PAGE the precursor migrates as a 90-110 kDa complex consisting of pro-TGF-β1 (30-390), the pro region of the precursor (30-278) and mature TGF-β1 (279-390) interlinked by disulfide bonds (Gentry et al., 1988, Mol. Cell Biol. 8, 4162). Three cysteine residues are located in the pro region of the precursor at amino acid positions 33, 223 and 225. Site-directed mutagenesis was used to change the Cys codons to Ser codons and mutant constructs were transfected into COS cells. Analysis of recombinant proteins by immunoblotting showed that compared to the wild type, the Ser-33 mutant yielded increased amounts of mature TGF-β1 dimer (24 kDa). This correlated with an approximately 4-fold increase in biological activity. Unlike the wild type, the mutant precursor proteins did not migrate as a single complex on non-reducing SDS-PAGE. Precursor- and mature-specific peptide antibodies recognized a 130-150 kDa species, while only precursor-specific antibodies detected a 75-85 kDa polypeptide. Ser-223 and Ser-225 mutants yielded near wild-type levels of mature TGF-β1. These precursor proteins migrated as a 90-110 kDa complex, but some monomeric precursor (44-56 kDa) was present, indicating that Cys-223 and Cys-225 may form interchain disulfide bonds. Thus, these three cysteine residues appear non-essential for production of biologically active TGF-β1 and Cys-33 in particular may have a regulatory role through interactions with other proteins.

E 106 BXH-2 MICE; A MODEL SYSTEM FOR MYELOID LEUKEMIA. Arthur M. Buchberg[1], Hendrick G.Bedigian[2], Nancy A.Jenkins[1], Neal G. Copeland[1]. [1]NCI-Frederick Cancer Research Facility, P.O. Box B, Frederick, MD 21701. [2]The Jackson Laboratory, Bar Harbor, ME 04609. BXH-2 mice are a recombinant inbred strain derived from C57BL/6J and C3H/HeJ mice. While both progenitor strains have low incidences of spontaneous leukemia, 100% of BXH-2 mice develop a granulocytic leukemia by 7-9 months. The occurrence of granulocytic leukemia appears causally associated with the expression of an ecotropic murine leukemia virus (MuLV). The structure of several known proto-oncogenes, growth factor loci and common integration sites that might be involved in BXH-2 myeloid leukemia were examined, by Southern blot analysis, to determine if they were sites of viral insertion in BXH-2 tumor tissue. These loci included ***Myc***, ***Pim-1***, ***Pvt-1***, ***Fms***, ***Trp53-1***, ***Fis-1***, ***Evi-1***, ***Myb***, ***Il-3***, ***Csfgm***, ***Csfg***, ***Csfm***, ***Fim-1*** and ***Fim-3***. No rearrangements were detected. These results imply that BXH-2 myeloid leukemogenesis is caused by viral activation of a novel set of proto-oncogenes. One somatically acquired provirus that we cloned from BXH-2 tumors identifies a common site of viral integration seen in 11% of the BXH-2 tumors analyzed. The locus, designated ***Evi-2*** (ecotropic viral integration site-2), has been mapped to mouse chromosome 11. Chromosome mapping revealed that ***Evi-2*** identifies a previously unmapped locus in the mouse potentially involved in myeloid leukemia. We identified two distinct transcripts of the ***Evi-2*** locus. Both transcripts are present in normal tissue, being most abundant in activated macrophages, brain and lung from adult mice. cDNA clones from normal brain have recently been isolated. Sequence data may provide insights into the function of ***Evi-2***. Research sponsored by the National Cancer Institute under contract NO1-CO-74101 with Bionetics Research, Inc.

E 107 TGF-BETA STIMULATES EXTRACELLULAR MATRIX-DEPENDENT STRATIFIED GROWTH OF CULTURED HUMAN FIBROBLASTS. Richard A.F. Clark, Georgia McCoy, and Joy Folkvord, Div. Dermatology, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO.
During wound repair, fibroblasts proliferate and migrate into a tissue defect until the defect is filled with stratified layers of cells and matrix. Such in vivo fibroplasia also occurs in many fibrotic disorders. Under normal tissue culture conditions human dermal fibroblasts (HDF) grow to confluence and then cease proliferation. We have delineated culture conditions that stimulate HDF to grow in stratified layers. When fresh medium with 10% fetal calf serum, 50 ug/ml ascorbate, and 300 uM proline were added 3 x/week to HDF harvested from neonatal foreskins, HDF proliferated and stratified 2-4 cell layers within the culture dish producing a tissue-like fibroplasia. Cell proliferation during this post-confluent growth was monitored by a Hoechst dye assay. The stratification was dependent on matrix production as anti-fibronectin antibodies (anti-FN) and ethyl 3, 4 dihydroxybenzoate (EDB), an ascorbate analogue, inhibited cell stratification and proliferation. These materials, at the same concentrations used in the HDF proliferation assays inhibited fibronectin and collagen matrix assembly as judged by immuno-fluorescence microscopy of HDF cultures using anti-fibronectin and anti-collagen antibody probes. Since TGF-beta is known to stimulate in vivo fibroplasia and is expressed in fibroblasts during the fibroplasia of wound repair, we examined the effect of TGF-beta on post-confluent growth. TGF-beta stimulated growth 172% of control. Stratification was increased to 4-8 cell layers. The TGF-beta effect was lost in the presence of anti-FN and EDB. Thus, TGF-beta appeared to stimulate post confluent HDF proliferation by inducing more matrix production. This culture system may prove valuable for investigating the regulation of wound repair and fibrotic disorders such as scirrhous carcinoma.

E 108 STIMULATION OF c-fos AND c-myc BY EPIDERMAL GROWTH FACTOR AND TRANSFORMING GROWTH FACTOR ALPHA OCCURS VIA A CALCIUM AND PROTEIN KINASE C INDEPENDENT PATHWAY. Anthony F. Cutry[1], Alan J. Kinniburgh[2], Daniel R. Twardzik[3], Michael J. Krabak[4], Sek-Wen Hui[4] and Charles E. Wenner[1], Departments of [1]Experimental Biology, [2]Human Genetics, and [4]Biophysics, Roswell Park Memorial Institute, Buffalo, NY 14263 and [3]Oncogen, Seattle, WA 98121.
We have previously described the induction of the c-fos and c-myc protooncogenes by epidermal growth factor (EGF) and transforming growth factor alpha (TGFα) in C3H 10T1/2 mouse embryo fibroblasts. In an effort to delineate the mechanism by which these two ligands (which both utilize the EGF receptor for signal generation) transduce a mitogenic signal from the cell membrane to the nucleus, we have investigated the role of calcium as well as that of the calcium and phospholipid dependent protein kinase, protein kinase C (PKC). We have found that down-regualtion of PKC by a 48h pretreatment with the phorbol ester 12-0-tetradecanoyl-13-phorbol acetate (TPA) has no effect on the ability of EGF or TGFα to induce the expression of fos and myc mRNAs. Rechallenging the cells with TPA cannot induce these mRNA species, indicating that the down-regulation of PKC was indeed complete. There may, in fact, be a slight potentiation of EGF/TGFα induction of c-fos and c-myc mRNAs in the PKC down-regulated system.
We have used the photoprotein aequorin to measure changes in intracellular calcium in 10T1/2 cells stimulated with EGF or TGFα. Both ligands are capable of inducing a significant transient increase in intracellular calcium in calcium containing media. However, when the same experiment is performed in calcium-free media, neither EGF or TGFα is capable of causing any detectable rise in intracellular calcium, indicating that the calcium transient is completely dependent on the presence of extracellular calcium. Parallel experiments were conducted to assay the effect of calcium-free media on the ability of EGF and TGFα to induce fos and myc mRNA expression. If a rise in intracellular calcium is involved in the stimulation of c-fos and c-myc mRNA expression by EGF/TGFα, then we should not see an increase in these mRNAs in calcium-free media. However, we have observed that calcium-free media has no effect on the accumulation of fos and myc mRNAs in response to EGF or TGFα. From these results, we have concluded that: 1)Both EGF and TGFα are equipotent mitogens in stimulating a rise in intracellular calcium, and this calcium transient is completely dependent on the presence of calcium in the extracellular medium; 2)The ability of EGF and TGFα to induce the expression of c-fos and c-myc mRNAs is the same in calcium-free media as it is in calcium supplemented media, and thus occurs via a calcium-independent mechanism; 3)PKC is not involved in the stimulation of these mRNA species, as indicated by phorbol ester induced down-regulation of PKC. Furthermore, in an effort to explain some of the "superagonist" properties of TGFα relative to EGF, it has been postulated that there is a bifurcation in the signalling pathways of the two ligands. From our data, it would appear that such a bifurcation, if it exists, does not occur during the "early" phase of mitogenic signalling.

E 109 DIFFERENTIAL INHIBITION OF TGF-β1 AND TGF-β2 ACTIVITY BY α_2-MACROGLOBULIN, David Danielpour and Michael B. Sporn, Laboratory of Chemoprevention, National Cancer Institute, Bethesda MD 20892.
Transforming growth factors-beta 1 and beta 2 (TGF-β1 and TGF-β2), two multifunctional modulators of cell growth, differentiation and numerous other cellular processes, are each highly conserved 25 kD homodimers that share 71% sequence homology and in most cases share similar or identical receptor binding and biological activities in vitro. TGF-β1, released during platelet degranulation, is complexed to serum α_2-macroglobulin (α_2M) by an unknown mechanism, resulting in a biologically latent form. Here we show that human, calf, fetal bovine and rabbit sera all block the binding of [^{125}I]TGF-β2 to receptors on A549 human lung carcinoma cells substantially better than the binding of [^{125}I]TGF-β1. Our results, which imply that α_2M is responsible for this difference in the ability of serum to block binding, are as follows: 1) Affinity labeling studies demonstrate that α_2M is the major serum binding protein for both TGF-β1 and TGF-β2; 2) Purified α_2M is 20-fold more potent in inhibiting the binding of TGF-β2 than that of TGF-β1 to receptors on A549 cells; 3) Purified α_2M (200 μg/ml) does not block TGF-β1 inhibition of CCL-64 mink lung cell growth, but reduces this activity of TGF-β2 10-fold. Our data suggest that α_2M is an important differential regulator of the biological activities of TGF-β1 and TGF-β2 in vivo.

E 110 **CLONING OF THE BREAKPOINT IN THE 10;14 TRANSLOCATION OF T-CELL NEOPLASIA AND IDENTIFICATION OF A CHROMOSOME 10 SPECIFIC SEQUENCE,** **Ian Dubé, Ming Lu, Mark Minden, Susana Raimondi[++], Huntington Willard and Andrew Carroll[+], The University of Toronto, 100 College Street, Toronto, Canada, M5G 1L5, The St. Jude Children's Research Hospital[++] and The University of Alabama Medical Center[+].** The t(10;14)(q24;q11) is a primary acquired translocation seen in the leukemic cells of about 10% of children with T-ALL. We performed Southern analysis of leukemic cell DNA from 7 such patients and found rearrangements within the TCR delta chain gene D-J subunits in 5. We made a mouse/human hybrid containing, as its only relevant human chromosome, the derivative 10 from one patient. Southern analysis of DNA from this hybrid enabled us to localize the breakpoint to a 3kb BamHI-HindIII fragment. We made a DNA library from leukemic cells from the above patient and isolated a phage containing a 13kb BamHI fragment that included 3kb of candidate chromosome 10 derived DNA. From this, we subcloned a 1.8kb sequence and demonstrated, by hybrid panel mapping, that it contained chromosome 10 derived DNA. This insert will be used to probe established T-cell lines and our own panel of somatic cell hybrids, made between mouse leukemic cells and human leukemic cells bearing the t(10;14), for hybridizing mRNA. cDNA libraries will be made from appropriate cell lines. Our immediate objective is to identify a DNA sequence containing at least some of the exons of the putative, new, cellular oncogene in chromosome 10q24.

E 111 **TRANSCRIPTIONAL REGULATION OF PROTO-ONCOGENE EXPRESSION BY EPIDERMAL GROWTH FACTOR, TRANSFORMING GROWTH FACTOR β1, AND TRIIODOTHYRONINE IN MDA-468 CELLS,** J. A. Fernandez-Pol[+*], P. D. Hamilton[+], V. M. Schuette[+] and D. J. Klos[+], Laboratory of Molecular Oncology, [+]VA Medical Ctr., and [*]St. Louis University, St. Louis, Mo. 63106
We have examined the epidermal growth factor(EGF)-dependence of the transcription of a variety of different proto-oncogenes, using cultured human mammary carcinoma MDA-468 cells and a nuclear run-on transcription assay. We found that stimulation of MDA-468 cells with EGF regulates the transcription of EGF-receptor(R) and c-erbB-2 proto-oncogenes. Furthermore, we provide evidence that transforming growth factor β1 (TGF-β1) modulates the EGF-dependent transcription of EGF-R and c-erbB-2 genes. We also found that T_3 (triiodothyronine) exerts synergistic control on the action of EGF alone, or in association with TGFβ1, on EGF-R and c-erbB-2 gene transcription. We established that after addition of EGF, cytoplasmic EGF-R mRNA levels are increased severalfold and that this accumulation is enhanced by the presence of TGF-β1 and/or T_3. Distinctively, retinoic acid treatment also enhanced the EGF-dependent expression of the EGF-R gene and acted either synergistically or antagonistically with TGFβ1 and/or T3. The results suggest that co-operative interactions among EGF-, TGF-β1-, and T_3-generated signals at the transcriptional level may mediate, at least in part, the combined actions of EGF, TGF-β1 and T_3 in target cells.

E 112 RETROVIRAL EXPRESSION OF TRANSFORMING GROWTH FACTOR ALPHA DOES NOT TRANSFORM FIBROBLAST OR EPITHELIAL CELLS, Eric Finzi, Jackie H. Pierce, Rik Derynck and Stuart A. Aaronson, Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland 20892 and Genentech, Inc., California 94080. Transforming growth factor alpha (TGFa), is a peptide which helps to impart anchorage-independent growth to normal rat kidney (NRK) cells in culture and is secreted by many rodent and human tumor cells. To directly investigate the transforming properties of this factor, we constructed a murine retrovirus which expresses the human coding sequence for TGFa. Infection of NIH 3T3 cells with the TGFa retrovirus led to the intregration of a transcriptionally active provirus and overexpression of biologically active TGFa, but failed to induce morphological transformation. Similarly, the TGFa retrovirus failed to induce morphological transformation of six other types of rodent fibroblasts. We also investigated the effect of TGFa expression on the growth of BALB/MK mouse keratinocytes which require epidermal growth factor (EGF) for proliferation. We show that exogenously added TGFa is an extremely potent mitogen for BALB/MK cells. However, retroviral expression of TGFa in BALB/MK cells failed to relieve dependence on exogenously added EGF or TGFa for cell growth. These results suggests that overexpression of TGFa does not, by itself, transform rodent fibroblasts or keratinocytes.

E 113 **A PROTEIN TYROSINE KINASE ACTIVATED BY AUTOCRINE LOOP IN A HUMAN GASTRIC CARCINOMA LINE.** Silvia Giordano, Maria Flavia Di Renzo, Radha Narshiman and Paolo M. Comoglio. Dept. of Biomedical Sciences & Oncology, University of Torino Medical School, 10126 Torino, Italy.

Antibodies to phosphotyrosine (P-Tyr) detected in "western" blot analysis a protein of 145,000 M_r which is phosphorylated at tyrosine in a gastric carcinoma cell line. In cells metabolically labelled with ^{32}P-ortophosphate this protein (p145) was found phosphorylated on tyrosine and on serine. P145 is a cystein-rich transmembrane glycoprotein with a domain exposed at the cell surface that can be labelled by ^{125}I, under non-permeating conditions, and that can be cleaved by mild trypsin treatment of intact cells. Electrophoretical migration performed under non-reducing conditions revealed a shift of p145 to an apparent M_r of 190.000 due to a covalently linked polypepdide chain of M_r50.000. After immunoprecipitation with P-Tyr antibodies, p145 displays a strong associated protein kinase activity "in vitro", becoming phosphorylated on tyrosine. No immunological cross-reactions, nor similarities in phosphopeptide patterns were found between p145 and other known tyrosine kinases. The kinase activity both "in vivo" and "in vitro" was unaffected by the addition of known growth factors. However, p145 was rapidly dephosphorylated "in vivo" when cells were exposed to low pH or treated with suramin, conditions known to dissociate ligands from their receptors. All together, these data suggest that p145 is associated with a protein tyrosine kinase which, in the tumor cell line studied, is activated through an autocrine loop mediated by an as yet unidentified factor.

E 114 THE PURIFICATION OF TGF-ß FROM HUMAN UMBILICAL CORD TISSUE, L.I. Gold, Dept. of Pathology, New York Univ. Med. Center, 560 First Ave. IRM806, New York, NY 10016

Transforming growth factor-ß (TGF-ß) polypeptides influence the proliferation and differentiation processes of cells, and specifically demonstrate growth inhibitory activity on a variety of cell tissues, including carcinomas.

We found that an acidic extract of human umbilical cord tissue possessed potent growth inhibitory activity on certain cell lines, including a human lung carcinoma (A549). This growth inhibitor was shown to migrate unreduced at 25 kDa by SDS-PAGE and to be related to TGF-ß by Western blot analysis. A novel purification protocol for TGF-ß was devised employing hydrophobic interaction chromatography (HIC) on phenyl-sepharose followed by two separate reverse phase HPLC steps, first using a C18 and then a C4 resin; both with an eluting mobile phase of acetonitrile. The first (HIC) and second step (C18) yielded a 40 and 32,000 fold increase in purification, respectively, and TGF-ß comprised approximately 60% of the total protein by the second step. The final, chromatographic step (C4) revealed two separate peaks of growth inhibitory activity. The more hydrophobic peak was TGF-ß-1 and the less hydrophobic peak of activity possessed 10-20 times higher specific activity than TGF-ß-1.

Employing the above described method of purification, human umbilical cord tissue appears to provide a good source of TGF-ß, yielding 10 µg per 250 grams wet weight (activity=100 units/ng). Studies are currently in progress to characterize the more hydrophobic eluting growth inhibitory protein, which possibly is a member of the TGF-ß family.

E 115 INVERSE RELATIONSHIP BETWEEN EGF-RECEPTOR AND TGF-ALPHA LEVELS IN HUMAN ORAL SQUAMOUS CELL CARCINOMAS. Martin R. Green[1] and Maxine Partridge[2]. [1]Unilever Research, Colworth Laboratory, Sharnbrook, Bedford, U.K. MK44 1LQ. [2]Charing Cross Sunley Research Centre, Hammersmith, London W6 8LW. We have examined a series of oral squamous cell carcinomas (SCC) for TGF-alpha transcripts, protein levels and for the expression of the EGF-receptor. TGF-alpha protein was measured by RIA of part-purified acid/ethanol tumour extracts, while EGF-receptor level was quantified using an immunohistochemical stain index (King et al). All tumours contained 4.5kb TGF-alpha transcripts,TGF-alpha protein (0.5-9ng/gm wet wt. of tissue) and stained for the EGF-receptor. Statistical analysis showed a highly significant inverse relationship between the log TGF-alpha concentration and the EGF-R stain index (-0.69 $p<0.02$) and a positive correlation between log TGF-alpha concentration and histological grade (0.694 $p<0.02$). All samples were negative for the 5.0kb preproEGF transcript, and the presence of TGF-alpha in tumour tissue further confirmed by immunohistochemistry. The data are consistent with an autocrine role for TGF-alpha in tumour growth and can be interpreted in terms of active production, secretion, internalisation and metabolism of TGF-alpha/EGF-receptor.
(King et al. 1985, Cancer Research 45, 5728)

E 116 TUMORIGENICITY IN NUDE MICE OF HUMAN BREAST CARCINOMA CELLS IS CORRELATED WITH THE LEVEL OF AMPLIFICATION OF C-MYC AND THE EXPRESSION OF TGFα.
Hédi HADDADA[1], Eliane LAZAR[1], Christian LAVIALLE[2], Nazanine MODJTAHEDI[2], Olivier BRISON[2] and Pierre MAY[1].
1 - I.R.S.C., B.P. n°8, 94802 Villejuif (France). 2 - I.G.R., 94805 Villejuif (France).

The transforming growth factors (TGFs) are believed to play an important role in determining some phenotypic characteristics of transformed cells and evidences have accumulated on their interactions with oncogenes. We examined the relationship between the level of amplification of c-myc oncogene and the secretion of TGFα by 7 different subclones of cells derived from a human breast carcinoma cell line, SW613-S. It has been reported (Lavialle et al., Oncogene, 3, 1988) that cells with high c-myc amplification (40 to 60 fold) were tumorigenic in nuce mice ; whereas, cells with low c-myc amplification (3 fold) were not tumorigenic. Our data showed that only tumorigenic cells with high c-myc amplification secreted significant amounts (8 to 20 ug) of TGFα in the medium after 48 h of culture as determined by radioimmunoassay and by stimulation of quiescent NRK cells. While, the TGFα receptor was down regulated in the secretory cells.

These data suggest that amplification of c-myc oncogene and overexpression of TGFα are two related events contributing to the oncogenic phenotype of the cells. However, we do not know yet whether TGFα expression in these cells is under control of c-myc (and vice versa) or not

E 117 ALTERED GROWTH REGULATION AND PROTEIN EXPRESSION IN TUMORIGENIC v-raf INFECTED RAT LIVER EPITHELIAL CELLS, Lori L. Hampton, Peter J. Worland, Anthony C. Huggett, Caroline P. Ford and Snorri S. Thorgeirsson, Laboratory of Experimental Carcinogenesis, NCI, NIH, Bethesda, MD 20892.
Rat liver epithelial (RLE) cells were infected with a retrovirus containing the v-raf oncogene (3611-MSV). The morphologically transformed colonies were inoculated into nude mice and a series of single cell clones, R3611T-1, 2, 3, 4, 5, and 7, were isolated from a single tumor. Southern analysis revealed two insertion sites for v-raf in clones R3611T-4 and 5 while the other clones had only one integration site. All of the clones expressed v-raf mRNA although the level of expression did not correlate with the number of insertion sites. In vitro morphology ranged from typical epitheloid to a more elongated, spindle shape with loss of contact growth inhibition. All of the clones had increased growth rates compared to the normal cells and were also more resistant to the growth inhibitory effects of TGF-ß1. High resolution two-dimensional gel analysis identified two high molecular weight proteins (pI/Mr 5.9-7.2/205K, 6.5-7.5/160K) present in the original cell population and in clones R3611T-4 and 5, but absent in R3611T-1, 2, 3, or 7. These protein changes correlated with the morphological appearance of the cells in culture. When reinjected into nude mice all of the clones were tumorigenic but with different latency periods. The clones with the more epitheloid morphology, R3611T-4 and 5, demonstrated a latency period of 7-10 days compared to only 2-4 days for R3611T-1, 2, 3, and 7. Histological examination of the resultant tumors presented a "mixed epithelial-mesenchymal" type, ranging from carcinoma-like for R3611T-4 and 5 to sarcoma-like for R3611T-1, 2, 3, and 7. The present data suggest that expression of two high molecular weight proteins is negatively associated with tumorigenicity and in vitro and in vivo morphology of transformed RLE cells.

E 118 RELEASE OF TRANSFORMING GROWTH FACTOR-α FROM A NONSECRETING HUMAN BREAST CELL LINE BY CATHEPSIN D, John L. Henry and Gregory S. Schultz, Department of Biochemistry, University of Louisville School of Medicine, Louisville, KY 40292. TGF-α has been detected in the conditioned medium of several human breast cancer cell lines and stimulates their growth by binding to TGF-α/EGF receptors on the cells by an autocrine/paracrine mechanism. TGF-α is synthesized as a 160 amino acid transmembrane precursor which contains in the extracellular domain the 50 amino acid form (50mer) that is present in conditioned medium. If the transmembrane precursor has low activity compared to the 50mer, then processing of the precursor by proteolytic cleavage is an important regulatory event in the autocrine mechanism. We have reported previously that the HBL-100 cell line does not secrete the 50mer into the conditioned medium but synthesizes immunoreactive TGF-α detected in solubilized membranes. Treatment of HBL-100 cells in chemically defined medium (CDM) with Cathepsin D, a protease induced by estrogen treatment of MCF-7 cells, produced a 20-fold increase in immunoreactive TGF-α in CDM. TGF-α material released by cathepsin D treatment competed for ^{125}I-EGF binding to TGF-α/EGF receptor. Incubation of HBL-100 cells with pepstatin A and cathepsin D significantly reduced the release of TGF-α into CDM. We are investigating if secretion of TGF-α 50mer can be inhibited by a synthetic peptide containing a noncleavable surrogant amide bond which mimics the ala-val cleavage sites at the N- and C- terminals of the 50mer. Processing of the transmembrane TGF-α precursor may be an important regulatory step in the autocrine mechanism of TGF-α stimulation of breast cancer cell growth.

E 119 EPITOPE MAPPING OF RAT AND HUMAN TRANSFORMNG GROWTH FACTOR α, P. D. Hoeprich, Jr., S. Martin, B. Langton, J. Jackson, C. Volin* and J. P. Tam*, Triton Biosciences, Alameda, CA 94501; *The Rockefeller University, New York, NY 10021. A panel of murine monoclonal antibodies and rabbit polyclonal antibodies were raised against rat and human transforming growth factor α. Continuous epitopes recognized by these antibodies were identified using two related methodologies. In one case, 22 synthetic peptides based on the hTGFα sequence consisting of 8 amino acids each and overlapping one another by 2 amino acids were prepared and tested for reactivity with the various antibodies. Secondly, the method of Geysen [Proc. Natl. Acad. Sci. USA 81, 3998-4002 (1984)] was used. Three sets of sequentially overlapping 4-mers, 6-mers and 8-mers based on the hTGFα sequence were prepared. Each of the antibodies was evaluated for reactivity by the peptide-pin based ELISA. By both techniques, the monoclonal antibodies defined two immunodominant regions spanning residues 2-8 and residues 22-29. The polyclonal antibodies gave a more heterogeneous response binding primarily to the same two epitopes defined by the monoclonal antibodies, as well as regions covered by residues 37-40 and to a lesser extent by residues 43-50.

E 120 ROLE OF A PROTEIN KINASE C (PKC) AND TRANSFORMING GROWTH FACTOR-β (TGF-β) INDUCED GENE IN CELL TRANSFORMATION. Johnson, Mark D.*, Housey, G.M., Kirschmeier, P.T., Hsiao, W.L. Wendy, and Weinstein, I.B. *Northwestern Univ., Chicago, IL, and Cancer Center, Columbia Univ., New York NY.
Constitutive over-expression of the β_I isoform of PKC activity has been shown to induce a transformed phenotype in fibroblasts. The mechanism of this effect may involve the altered regulation of specific PKC-responsive genes. Phorbin is a PKC-inducible gene (MCB 7:2821, 1987) that has been shown to be induced by a wide range of mitogens, including PDGF, FGF, EGF, serum and also TGF-β, which showed a strong induction of phorbin expression that was synergistic with other growth factors. The role of phorbin in the induction of transformation by PKC is suggested by the fact that overexpression of the phorbin cDNA, using a retroviral expression vector, will also induce transformation in fibroblasts. The relative RNA levels expressed by individual clones correlates with their efficiency of colony formation in soft agar (up to 98% cloning efficiency with the highest expressors). The addition of concentrated, conditioned serum-free medium from high expressing cells induces anchorage-independent growth in NIH/3T3 and NRK cells, suggesting that the induction of the transformed phenotype in over-expressing cells is mediated through an autocrine mechanism. This mechanism is supported by evidence from other laboratories that have demonstrated a high-affinity membrane receptor for the human analogue of the phorbin protein. Therefore, the phorbin gene product may play a critical role in mediating the transforming activity of PKC and TGF-β. (Research supported by NCI grants CA 02656 and CA 07870).

E 121 REGULATION OF SQUAMOUS CELL CARCINOMA CELL GROWTH BY TYPE ß TRANSFORMING GROWTH FACTOR AND EPIDERMAL GROWTH FACTOR, Shawn Jones, Glen Franklin, Cynthia Greaton, Serge Martinez and Gregory Schultz, Departments of Biochemistry, Anatomy, and Division of Otolaryngology, University of Louisville School of Medicine, Louisville, KY 40292. Growth of human head and neck squamous cell carcinomas (SCC) may be regulated, in part, by an imbalanced autocrine mechanism involving stimulatory factors (TGF-α/EGF), inhibitory factors (TGFß) and their receptors. We have demonstrated previously that many human epidermoid tumors and SCC cell lines of the head and neck express elevated levels of the TGF-α/EGF receptor, respond mitogenically to EGF, and synthesize and secrete TGF-α and EGF. To determine if human head and neck SCC cell lines retain the normal inhibitory response to TGFß, effects of various concentrations of TGFß were studied on six human SCC cell lines and normal human keratinocytes. Concentrations of TGFß (0.1 ng/ml to 10 ng/ml) significantly ($p<.002$) suppressed serum-stimulated mitogenesis in keratinocytes from 40% to 95% respectively. In contrast, none of the SCC cell lines tested demonstrated a significant decrease in serum-stimulated DNA synthesis across the same range of TGFß concentrations. TGFß significantly ($p<0.01$) inhibited EGF-induced DNA synthesis at high levels of EGF when FaDu or Detroit 562 cells were cultured in chemically defined media without serum (CDM) by 17% at 1.0 nM and 57% at 10 nM of EGF. However, TGFß significantly ($p<.001$) enhanced EGF-induced DNA synthesis by ≈35% at 0.1 nm EGF. Specific binding of ^{125}I-TGFß was detected in normal keratinocytes and SCC cell lines at comparable levels ranging from 3,000 to 30,000 receptors/cell. Thus, malignant growth of SCC may be due to a combination of excessive stimulation by autocrine growth promoting factors and their receptors and a loss of sensitivity to autocrine growth inhibition.

E 122 BIPHASIC APPEARANCE OF TRANSFORMING GROWTH FACTOR-β IN WOUND HEALING. Cynthia JM Kane*, Philip C Hanawalt*, A Merrill Knapp# and Jonathon N Mansbridge#. *Department of Biological Sciences, Stanford University, and the #Psoriasis Research Institute, Palo Alto, CA 94305.

The effect of transforming growth factor-β [TGF-β] on cultured human epidermal keratinocytes is consistent with a role for TGF-β in the physiological regulation of epidermal growth and differentiation. The proliferation of cultured keratinocytes is reversibly inhibited by TGF-β at picomolar concentrations. Cultured keratinocytes are induced by TGF-β to switch from normal differentiation to a regenerative maturation pathway, as determined by induction of keratins 6 and 16 synthesis and suppression of keratins 1 and 10 synthesis. These effects are consistent with the temporal and spatial expression of TGF-β during epidermal injury and wound healing as determined by immunohistochemical techniques. TGF-β was not detected in normal human epidermis; however, two peaks of TGF-β protein expression were defined in injured human skin. Specific anti-TGF-β antibody [gift of L. Ellingsworth] binding was detectable within the first few cells surrounding a site of injury within 5 minutes and was maintained for 5-10 minutes. This may represent a rapid first-phase proteolytic activation of latent, constitutively-expressed TGF-β protein due to the release of proteases at the site of injury. The induction of antibody reactive TGF-β was blocked by injury in the presence of protease inhibitors or formaldehyde; consistent with reports that the anti-TGF-β antibody designated CC detects primarily activated TGF-β protein. A second stronger induction of TGF-β protein was evident by 24 hours postinjury in all layers of the epidermis extending at least 1 mm from the injury. This later phase of TGF-β expression may be due to induction of TGF-β mRNA and protein synthesis resulting from the early activation of latent TGF-β, since TGF-β induces synthesis of its own mRNA. The presence of TGF-β in the basal layer of keratinocytes on day 1, when proliferation of these cells is transiently inhibited following injury, is consistent with the ability of TGF-β to reversibly inhibit cell proliferation. By day 3 postinjury, TGF-β was present at very high levels suprabasally but was markedly absent from the basal layer in which cell proliferation was again apparent. Thus normally differentiating skin does not exhibit detectable TGF-β, whereas skin induced to regenerative differentiation by injury exhibits a rapid activation of TGF-β followed by a prolonged induction and expression of TGF-β in the epidermal region surrounding an injury.

E 123 TRANSFORMING GROWTH FACTOR ß1 AND GRANULOCYTE/MACROPHAGE COLONY STIMULATING FACTOR ACT TOGETHER TO PROMOTE GRANULOCYTE GROWTH AND DIFFERENTIATION Of NORMAL MURINE HEMATOPOIETIC BONE MARROW CELLS. J.R. Keller[1], L.R. Ellingsworth[2] and F.W. Ruscetti[3]. [1]BCDP-P.R.I. and [3]LMI-BRMP, FCRF-NCI, Frederick, MD 21701, [2]Collagen Corp., Palo Alto, CA 94303. We have shown that transforming growth factor-ß1 (TGF-ß1) acts as a selective inhibitor of hematopoietic cell growth. In particular, TGF-ß1 inhibits Interleukin-3 induced immature hematopoietic colony formation in soft agar (CFU-GEMM) while more mature lineage restricted colony formation is unaffected. In contrast, TGF-ß1 in the presence of saturating concentrations of GM-CSF promotes a 3-5 fold increase in large colony formation as well as increase in total cells in a dose dependent manner with an ED_{50} of 10 to 20 pM, while TGF-ß1 alone exerts no effect. Morphological and histochemical examination of colonies in soft agar demonstrate that the colonies consist of large numbers of differentiated neutrophils. Suspension cultures show a similar increase in cell number and neutrophilic differentiation as well as decreased numbers of monocytes and macrophages. Last, limit dilution analysis demonstrate a 2-5 fold increase in cells that respond to GM-CSF plus TGF-ß1 versus GM-CSF alone. This effect is not observed with other CSF's, thus we propose that TGF-ß1 and GM-CSF act in a two signal model to stimulate granulopoiesis as measured by large granulocyte colony formation, tentatively designated burst forming unit-granulocyte (BFU-G).

E 124 CHARACTERIZATION OF THE PROMOTER REGION OF THE TGF-β1 GENE REPONSIVE TO TGF-β1 AUTOINDUCTION. Seong-Jin Kim, Kuan-Teh Jeang*, Adam Glick, Michael B. Sporn and Anita B. Roberts. Laboratory of Chemoprevention, National Cancer Institute and Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases*, Bethesda, MD 20892.

The promoter region of the TGF-β1 gene was analyzed by S1 mapping and primer extension. Multiple transcriptional start sites were identified. To determine the location of sites that may be important for the function of the promoter of TGF-β1, we joined the sequences upstream of the first major transcriptional start site and between two major transcriptional start sites of the TGF-β1 gene to the coding region for chloramphenicol acetyltransferase (CAT). The chimeric genes produced high levels of CAT activity in A-549 (human lung carcinoma) cells. Sequences located between nucleotides -454 to -323 and between the two major transcriptional start sites have positive regulatory activities and are induced by TGF-β1. The CAT activity of the upstream human TGF-β1 promoter-CAT gene is increased 8 to 10-fold by treatment of cells with TGF-β1, whereas that of the second promoter is increased approximately 3 to 4-fold. Using an S1 nuclease protection assay of CAT mRNA, we found that the steady-state expression of CAT mRNA also is markedly increased. Seven distinct factors present in nuclear extracts from A-549 cells interact with the sequences between -454 to -323, strongly supporting the involvement of sequence-specific transcription factors in the transcriptional auto-activation of the human TGF-β1 gene.

E 125 GROWTH INHIBITION OF HUMAN PROSTATE CANCER CELLS BY SURAMIN. Cornelius Knabbe, *Gerhard Zugmaier, Udo Kellner and *Anton Wellstein, Dep.Clin.Chem., Med.Univ.Clinic, D-2000 Hamburg 20, Fed.Rep.of Germany, and *Lombardi Cancer Center, Georgetown University Medical Center, Washington, D.C. 20007.

It has been previously shown that estrogens might exert their action on human breast cancer cells through coordinated control of secreted growth factors which function in an autocrine/paracrine fashion. We now demonstrate that androgens might regulate growth of androgen-responsive human prostate cancer through a similar mechanism. Human prostate cancer cell lines in culture secrete a biologically active TGFα species, possess high affinity EGF-receptors and show an increased growth rate under treatment with EGF/TGFα. Secretion of TGFα can be induced by androgens in the androgen responsive human prostate cancer cell line LNCaP. The polyanionic compound suramin which has ben shown to inhibit the action of a variety of growth factors including TGFα inhibits growth of human prostate cancer cell lines in a dose dependent and reversible fashion. Growth inhibition by suramin can be partially reversed by the simultaneous addition of androgens. These observations suggest the existence of at least two types of mechanisms by which androgens regulate growth of human prostate cancer. These mechanisms can be distinguished by their different sensitivity to suramin.

E 126 SELECTIVE DEGRADATION OF TRANSFORMING GROWTH FACTOR- ALPHA BY PANCREATIC CANCER CELLS, Murray Korc, Departments of Medicine and Biochemistry, University of Arizona, College of Medicine, Tucson, AZ 85724

T_3M_4 human pancreatic carcinoma cells avidly bound and internalized ^{125}I-labeled epidermal growth factor (EGF), but did not readily degrade the ligand. Pulse-chase experiments in which the cell-bound radioactivity was allowed to dissociate into the incubation medium in the presence of unlabeled EGF, indicated that the majority of the released ^{125}I-EGF consisted of intact EGF and a slightly processed species that readily bound to the cell. Omission of unlabeled EGF during the chase period markedly decreased the amount of radioactivity in the incubation medium, mainly as a result of the rebinding of EGF to the cells. In contrast, T_3M_4 cells readily degraded ^{125}I-labeled transforming growth factor-α (TGF-α), and the released radiolabeled products did not rebind to the cells. Degradation of both factors was almost completely blocked by the lysosomotropic compound methylamine. ASPC-1 human pancreatic carcinoma cells also failed to extensively degrade ^{125}I-EGF, but markedly degraded ^{125}I-TGF-α. Immunoprecipitation of the EGF receptor with specific polyclonal antibodies and Western blot analysis revealed the anticipated 170 kDa protein in T_3M_4 cells. Both EGF and TGF-α enhanced EGF receptor phosphorylation, degradation, and down regulation. However, TGF-α was less effective than EGF with respect to all three actions. These findings suggest that the rapid processing of TGF-α and the attenuated ability of this factor to induce EGF receptor degradation and down regulation in T_3M_4 cells may be due to differences between the 2 ligands with respect to EGF receptor activation.

E 127 TGFβ SUPPRESSES THE INVASIVENESS OF TUMOR CELLS. Shunichiro Kubota, Reuven Reich, Bruno Clement, Yoshihiko Yamada, and George R. Martin. Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, NIH, Bethesda, Maryland 20892.
Basement membranes are a thin extracellular matrices which surround epithelial and endothelial cells and contain a unique set of proteins including laminin and collagen IV. Basement membranes create a major barrier for the invasion process in tumor cell metastasis. To metastasize, tumor cells must attach to basement membranes, degrade the matrix and migrate through. The interaction of tumor cells with basement membranes induce the production of collagenase IV needed to digest and invade basement membranes. Malignant tumor cells are known to have increased laminin recptors, and be more migratory, and are able to form branching colonies on reconstituted basement membrane and invade through it. In this report, we have examined whether TGFβ changes the invasive phenotype of malignant tumor cells. TGFβ decreased the levels of laminin receptors and collagenase IV activity in human fibrosarcoma cells (HT-1080) and murine melanoma cells (B16-F10). TGFβ treatment also reduced the chemotactic activity of these cells in a Boyden-chamber assay. HT-1080 cells form branching structures when plated on a petri dish coated with a reconstituted basement membrane (matrigel). TGFβ inhibited the branch formation of HT-1080 cells. Furthermore, TGFβ in a dose-dependent manner reduced the invasiveness of HT-1080 cells and B16-F10 cells through basement membranes in a chemoinvasion assay in vitro. These results suggest that the pleiotropic effects of TGFβ reduced the invasiveness of malignant cells.

E 128 GROWTH INHIBITION OF HUMAN MAMMARY TUMOR CELL LINES BY TRANSFORMING GROWTH FACTOR BETA-1: INTERACTION WITH EGF AND AMILORIDE, Willy Küng, Eva Silber and Urs Eppenberger, Department of Research and Department of Gynecology, Kantonsspital Basel, CH-4031 Basel, Switzerland
Transforming growth factor beta-1 (TGF β-1) inhibited under serum-free conditions the proliferation of the human mammary tumor cell line ZR-75-1 at concentrations higher than 10^{-11}M. The stimulatory effects of EGF on these cells are inhibited by TGF β-1 and nearly blocked at high (nanomolar) concentrations.
40 S ribosomal protein kinase S6, which is activated by EGF in ZR-75-1 cells is not responding to TGF β-1, and does also not alter the kinase activation induced by EGF. Amiloride, a blocker of the Na^+/H^+ antiport, inhibited the proliferation of ZR-75-1 cells in a dose dependent manner (ED_{50} = 50 μM). Growth stimulation by EGF was partially neutralized by amiloride, which on the other hand intensified the growth inhibiting effects of TGF β-1.
The growth of the estradiol- and growth factor-independent cell line MDA-MB-231 is inhibited by TGF β-1, but even at high concentrations, TGF β-1 is not very effective. Interactions with EGF are marginal. Amiloride however, inhibited the growth of MDA-MB-231 cells in a way comparable to its effects on ZR-75-1 cells. Treatment of MDA-MB-231 cells with combinations of amiloride with TGF β-1 has only small synergistic effects.
This work was supported by the Swiss National Science Foundation and the Swiss Cancer Ligue.

E 129 TRANSFORMING GROWTH FACTOR-α : AN AROMATIC SIDE-CHAIN AT POSITION 38 IS ESSENTIAL FOR BIOLOGICAL ACTIVITY.
E. Lazar[1,2], E. Vicenzi, E. Van Obberghen-Schilling[3], B. Wolff, S. Dalton, S. Watanabe and M.B. Sporn. 1- NCI, NIH, Bethesda, Md 20892 ; 2- IRSC, BP 8, 94802 Villejuif, France ; 3- Centre de Biochimie, 06034 Nice, France.
Site-directed mutagenesis has been performed in the sequence of the fully processed, 50-amino acid human TGF-α . Deletion and substitution of amino acids located in the third disulfide loop, between cysteines 34 and 43, and disruption of disulfide bonds 8-21 and 34-43, resulted in loss of binding and colony-forming abilities. Mutation of tyrosine 38, a highly conserved amino acid in the family of EGF-like peptides was attempted. When tyrosine 38 is deleted, or mutated into alanine, serine, threonine or histidine, the mutant proteins are inactive. In contrast, when tyrosine is mutated into phenylalanine or tryptophane, biological activity is retained. The presence of an aromatic side-chain at position 38 of TGF-α seems to be essential for maintaining the structural conformation or for allowing the binding of TGF-α to its receptors.

E 130 COMPLEMENTARY DEOXYRIBONUCLEIC ACID CLONING OF CHICKEN TRANSFORMING GROWTH FACTOR-BETA MESSENGER RIBONUCLEIC ACIDS, Robert J. Lechleider, Sonia B. Jakowlew, Pamela J. Dillard, John P. Sverha, Michael B. Sporn and Anita B. Roberts, Laboratory of Chemoprevention, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Transforming growth factor-beta 1 (TGF-beta 1) cDNA has been cloned from a number of mammalian species including human, murine, bovine, porcine and simian. We have recently cloned a cDNA representing chicken TGF-beta 1 and find the mature coding portion of the deduced peptide to be 100% conserved with that of human TGF-beta 1. In addition to the chicken TGF-beta 1 mRNA, we have detected two additional distinct TGF-beta mRNAs of approximately 1.7 and 3 kb in cultured chicken embryo chondrocytes. We present the cDNA cloning, sequencing and expression of two new chicken TGF-betas, which we call TGF-betas 3 and 4 that show 72% and 82% identity, respectively, with the amino acid sequence of mature TGF-beta 1, and 76% and 64% identity, respectively, with the amino acid sequence of mature TGF-beta 2. Expression studies of TGF-betas 3 and 4 mRNAs during chicken embryo development are presented.

E 131 **TGF-*β* STRONGLY INDUCES PROLONGED SECRETION OF BOTH MEP AND p45, AN EXTRACELLULAR PROTEIN ASSOCIATED WITH REVERSION OF *ras*-TRANSFORMATION, IN NIH3T3 CELLS.** Brian J. Ledwith, Sujata Manam, Warren W. Nichols, and Matthews O. Bradley, Merck Sharp & Dohme Research Laboratories, West Point, PA 19446.

Antisense-*fos* RNA caused a dramatic reversion of the neoplastic phenotypes induced by the EJ-c-Ha-*ras* gene in NIH3T3 cells. Reversion by antisense-*fos* RNA in EJ cells had differential effects on the secretion of MEP, the 'major excreted protein' induced by *ras*-transformation, and p45, a protein whose secretion was inhibited by *ras*-transformation. Both MEP and p45 secretion are transiently induced by serum, PDGF, and TPA in NIH3T3 cells. The secretion of both proteins reaches a maximum about 2 to 4 hours following growth factor stimulation and then rapidly subsides. Transformation by EJ-*ras* caused the continuous over-secretion of MEP and the complete disappearance of p45 secretion, even after growth factor stimulation. Antisense-*fos* RNA in EJ cells did not alter the over-secretion of MEP, but caused a dramatic restoration of p45 secretion and its transient inducibility by growth factors. We examined the effects of TGF-*β* on extracellular protein secretion and found it was by far the most potent inducer of MEP and p45 secretion in NIH3T3 cells. Furthermore, TGF-*β* -induced secretion of MEP and p45 was extremely prolonged, persisting for more than 24 hours. Again, TGF-*β* -induced p45 secretion was inhibited by *ras*-transformation but restored by antisense-*fos* RNA. Thus, MEP and p45 appear to be coordinately regulated in NIH3T3 cells, but oppositely affected by *ras*-transformation; and *ras*-inhibition of p45 secretion appears to be mediated by c-*fos*, whereas *ras*-stimulation of MEP secretion does not appear to involve c-*fos*.

E 132 SITE-DIRECTED MUTAGENESIS OF THE TGFα PROTEOLYTIC CLEAVAGE SITES: EVIDENCE FOR INTERACTION BETWEEN THE INTEGRAL MEMBRANE FORMS OF THE TGFα PRECURSOR AND EGF RECEPTOR IN INTACT CELLS, David C. Lee, Sharon T. Wong, Lisa F. Winchell, Bryan K. McCune, Diana Gilligan, Joaquin Teixido, Joan Massague, Brian Herman and H. Shelton Earp, Lineberger Cancer Research Center, U. of North Carolina School of Medicine, Chapel Hill, NC 27514

The 50-amino-acid form of TGFα is cleaved from a conserved integral membrane glycoprotein by a protease that, in many tumor cells, appears to be limiting. To test whether the integral membrane precursor has biological activity in the absence of processing, we introduced amino acid substitutions at the proteolytic cleavage sites. Baby hamster kidney (BHK) cells transfected with expression vectors containing these altered sequences do not secrete detectable levels of the mature growth factor into the medium, but do express high levels of proTGFα at the cell surface. Co-culture of these BHK cells with A431 cells demonstrates that the integral membrane proTGFα may bind, and induce autophosphorylation of, EGF receptors on the surface of contiguous cells. In addition, there is a rapid rise in the A431 intracellular free Ca^{2+} level. These results indicate that proTGFα can be biologically active in the absence of processing, a fact that may also have implications with respect to the precursors of related growth factors.

E 133 EXPRESSION AND CHARACTERIZATION OF RECOMBINANT TGF-β2 PROTEINS PRODUCED IN MAMMALIAN CELLS, L. Madisen, M.N. Lioubin, A.L. Farrand, and A.F. Purchio, Oncogen, 3005 First Avenue, Seattle, WA 98121. Transforming growth factor β1 (TGF-β1) and β2 (TGF-β2) are two members of a family of growth differentiation factors that share obvious functional and structural similarities. Both TGF-β1 and TGF-β2 have been shown to exhibit a wide range of biological effects and, along with the more recently described TGF-β3, appear to be synthesized as large precursor proteins, the carboxy terminus of which comprises the mature TGF-β molecule. To study the relationship between these precursor proteins and their mature products, recombinant DNA plasmids encoding TGF-β1, TGF-β2 and a hybrid molecule TGF-β1(NH2)/β2(COOH) in which the amino terminal precursor portion of TGF-β1 was linked in phase to the carboxy terminus of mature TGF-β2 were constructed and transfected into COS cells. All three plasmids directed the synthesis of transforming growth factor which became bioactive upon acid activation. Immunoblots using site-specific anti-peptide antibodies demonstrated the secretion of both precursors and mature growth factors into COS supernatants. A stable Chinese Hamster Ovary (CHO) cell line expressing the hybrid TGF-β1(NH2)/β2(COOH) protein was isolated and was shown to secrete both precursor and mature forms of TGF-β1(NH2)/β2(COOH). Biological activity was demonstrated following acid activation. Protein sequence analysis of recombinant TGF-β2 produced by this CHO clone indicated that correct proteolytic cleavage had occurred suggesting that the processing signals contained within the TGF-β1 amino portion can function in producing mature TGF-β2. Receptor binding studies showed that TGF-β2 specifically bound predominantly to type III receptors on the surface of human palatal mesenchymal cells. The availability of active TGF-β2 should aid in determining its potential therapeutic use as a growth modulator.

E 134 RECOMBINANT TGF-β1 AND RETINYL ACETATE ACT IN A SYNERGISTIC MANNER TO CONTROL THE PROLIFERATION OF HUMAN LUNG CARCONOMA CELLS (A549) AND FETAL MINK LUNG CELLS (MV-1-Lu) IN VITRO, JoAnne Marzowski and Lisa B. Knox, Oncogen, 3005 First Avenue, Seattle, Washington 98121

Recombinant TGF-β1 induced growth inhibition of fetal mink lung cells (MV-1-Lu) and human lung carcinoma cells (A549) was measured in the presence of 10^{-9}M to 5 x 10^{-13}M retinyl acetate. There was no growth inhibition in either of the cell lines when treated with these concentrations of retinyl acetate alone. At concentrations of TGF-β1 (6-32 pg/mL) which show minimal growth inhibition, there is a significant synergistic effect produced by concurrent treatment with 10^{-11}M retinyl acetate. Binding assays were performed with ^{125}I-TGF-β. Pretreatment of both cell lines with 10^{-9}M retinyl acetate resulted in significant changes in both receptor number and affinity.

E 135 CHARACTERIZATION OF THE LYL-1 GENE: A CANDIDATE PROTO-ONCOGENE IN ACUTE LYMPHOBLASTIC LEUKEMIA. Julia D. Mellentin, Stephen D. Smith and Michael L. Cleary, Department of Pathology, Stanford University Medical Center, Stanford, CA 94305-5324.

We have cloned and characterized a novel candidate proto-oncogene which lies at the site of a t(7;19)(q34;p13) chromosomal translocation in T-cell ALL. Breakpoint DNA was cloned using hybridization probes for the beta T cell receptor locus (TCR) on 7q34. Sequence analysis of the breakpoint showed that this translocation joined chromosome 19 DNA to TCR joining segment J β1.1 and may have occurred as an error in TCR gene rearrangement. A 1.5 kb transcript, designated lyl-1, was identified in several hematolymphoid cell lines using chromosome 19 probes near the breakpoint.

cDNA cloning and sequencing studies showed that the lyl-1 transcript contains an open reading frame encoding a predicted 267 amino acid polypeptide. It is rich in basic amino acids and has 31% identity with the myc family of proteins over a 53 a.a. region directly N-terminal to the myc leucine zipper. The genomic configuration for this gene also showed a structural similarity to the myc genes in that the open reading frame extends 5' of the proposed ATG initiation site in exon 2 to an in frame CTG codon in exon 1, which may serve as an alternative translation initiation codon. In vitro translation experiments resulted in two major bands on SDS-PAGE and suggest that like myc the lyl-1 gene may code for two overlapping proteins differing at their amino termini. Structural studies demonstrated that the t(7;19) occurred in the first intron of the lyl-1 gene, resulting in its "decapitation" and juxtaposition with the beta TCR gene in a head-to-head configuration. The lyl-1 gene is transcriptionally disrupted by the t(7;19), leading to the synthesis of truncated mRNAs that can potentially code for only the protein that initiates at the ATG codon in exon 2.

E 136 LATENT FORM OF TGF-ß1: INTERACTION BETWEEN TGF-ß1 AND CARBOHYDRATE STRUCTURES IN THE N-TERMINAL PRECURSOR RENDERS IT LATENT. Kohei Miyazono and Carl-Henrik Heldin. Third Dept. of Intern. Med., Univ. of Tokyo, Hongo, Tokyo 113, Japan and Ludwig Institute for Cancer Res. Box 595, Biomedical Center, S-751 23 Uppsala, Sweden.
Latent form of TGF-ß1 (L-TGF-ß1) from human platelets contains one dimeric TGF-ß1 molecule, which is noncovalently associated with disulphide-linked complex of N-terminal remnant of the precursor and a single molecule of TGF-ß1 binding protein. Carbohydrate structures in the remnant of the TGF-ß1 precursor was found to be important to render TGF-ß1 latent. Treatment of L-TGF-ß1 with endo F led to the activation when analyzed by inhibition of endothelial cell growth and radioreceptor assay using NRK-49F cells. Analysis by SDS-gel electrophoresis and silver staining revealed that the remnant of the TGF-ß1 precursor was progressively degraded from 40 kDa to 31 kDa, whereas other components were not affected by endo F. Sialic acid and mannose-6-phosphate could also activate L-TGF-ß1, indicating that both of these monosaccharides might release active TGF-ß1 from latent complex by competing with the carbohydrate structures in the remnant of the precursor. Treatment with sialidase was also found to activate L-TGF-ß1. Thus, sialic acid and mannose-6-phosphate of Asn-linked carbohydrate complexes of the remnant of the TGF-ß1 precursor participate in the binding of TGF-ß1 in the latent complex and make it inactive.

E 137 PHOSPHORYLATION AND EXPRESSION OF A TRANSMEMBRANE PROTEIN IN DIVIDING AND QUIESCENT HODGKIN'S DISEASE CELLS. John F. Nawrocki, John S. Bennett, Erek S. Kirsten, and Richard I. Fisher, Section Hematology/Oncology, Loyola University Medical Center, Maywood, Il. 60153, and Hines VA Hospital, Hines, Il. 60141.
A study was done to identify a possible growth related function for a transmembrane phosphoprotein, called the HeFi-1 reactive membrane protein (HRMP), that is expressed in (1) Reed-Sternberg but not the normal inflammatory cells of Hodgkin's disease nodes, (2) select human hematopoietic tumor cell lines, and (3) EBV transformed cell lines. Phosphorylation and expression of the HRMP was analyzed in line L428 Hodgkin's disease cells that were either in their log phase of growth or made quiescent by removal of fetal calf serum. Regardless of the growth status of the cells, the HRMP was phosphorylated at both serine and tyrosine residues, even in cells that had been held quiescent up to 6 days and which demonstrated over 95% viability. Phosphorylation of the HRMP in serum starved cells was not reduced when cells were washed periodically to minimize the contribution of an autocrine factor. The level of HRMP synthesis, as measured by the incorporation of 35S-methionine, was the same for quiescent and dividing cells, and the half life of the protein was between 24 and 36 hours. In contrast, cells made quiescent by limiting their density and which had poor viability failed to express the phosphorylated transmembrane HRMP but did express its cytoplasmic precursor. We hypothesize that the HRMP may normally function as a competence related growth factor receptor and that its constitutive expression and phosphorylation contributes to the transformed phenotype of the tumor cell. We are testing this hypothesis and whether the HRMP is a novel oncogene product expressed in some human malignancies.

E 138 **IDENTIFICATION OF A MITOGENIC TUMOR MARKET IN BREAST MILK,** Janice Ness, Ph.D. and David Tapper, M.D., Department of Surgery, Children's Hospital and Medical Center, Seattle, WA 98105
Growth factors capable of stimulating DNA synthesis and cell division in cultured cells have been isolated from human, bovine, sheep and mouse milk. We have identified a new polypeptide growth factor in the milk of a substrain of mice with a high incidence of developing mammary tumor. This milk growth factor (MGF), absent in milk of mice which do not develop mammary tumors, is present prior to the appearance of a palpable tumor and is predictive of tumor development. Initial characterization has shown MGF to be a 15-18 kD cationic polypeptide mitogen which is acid- and heat-stable. A similar, if not identical, polypeptide mitogen has been identified in mouse mammary tissue, human breast cancer tissue and human breast cyst fluid. A preliminary study indicates that the level of this mitogen in human breast cyst fluid is directly related to the increasing risk of breast cancer. Purification and peptide sequence analysis of MGF is in progress.

E 140 PURIFICATION AND AMINO ACID ANALYSIS OF TRANSFORMING GROWTH FACTOR TYPE e, Pamela G. Parnell, John Wunderlich, Bobbie J. Carter and Jaroslava Halper, Departments of Veterinary Pathology and Chemistry, University of Georgia, Athens, GA 30602. We report on purification of TGFe, a novel transforming growth factor, from bovine kidney and its amino acid analysis. TGFe is a 22 K polypeptide which stimulates anchorage independent growth of certain epithelial cells (Cancer Res. 47:4552, 1987). SW-13 cells, derived from a human carcinoma of the adrenal cortex, serve as indicator cells for this growth factor. Initial acid-ethanol extraction of bovine kidney (ED_{50}40µg) was followed by batch ion exchange chromatography using Bio Rex 70 resin. The activity eluted with 1 M ammonium acetate was concentrated and diafiltered on an Amicon concentrator. The concentrate was purified on a molecular sieve Bio-Gel P-60 column eluted with 1 M acetic acid (ED_{50}0.3µg) and then on Heparin Sepharose affinity column. Active fractions were further separated on HPLC using sequential reverse phase C_8 and C_{18} columns eluted with linear (25-40%) acetonitrile gradient in 0.1% TFA. Final purification to homogeneity was achieved by TGFe elution from an SDS-polyacrylamide gel. The protein was electrophoretically bound to a PVDF membrane and subjected to amino acid analysis. TGFe appears to be composed of 168-178 amino acids. The major amino acids are lysine, glutamic acid/glutamine, aspartic acid/asparagine and glycine. The protein has low relative contents of aromatic acids which we suspected because of lack of absorption at 280 nm. The high content of aspartic acid/asparagine indicates TGFe is a glycoprotein.

E 141 DENSITY-DEPENDENT INHIBITION OF CELL GROWTH BY TRANSFORMING GROWTH FACTOR-β1 IN NORMAL HUMAN FIBROBLASTS, Ylva Paulsson, M. Patricia Beckmann, Bengt Westermark AND CARL-HENRIK HELDIN, Department of Pathology, University Hospital, S-751 85 Uppsala, Sweden.
TGF-β consists of two chains, β1 and β2, which form homo- or heterodimers, that bind to specific receptors found on almost all cell types. Depending on cell types and culture conditions, TGF-β has been found to either inhibit or stimulate cell proliferation. We report here that TGF-β1 inhibits platelet-derived growth factor (PDGF)-induced DNA synthesis in normal human fibroblasts in a cell density-dependent manner; no inhibition was seen in sparse cultures, approximately 50% inhibition in confluent cultures, and an almost total inhibition in dense cultures. The PDGF-inducible genes c-*myc* and c-*fos* were induced also by TGF-β1. Simultaneous addition of TGF-β1 and PDGF resulted in sustained, rather than transient, expression of c-*fos* mRNA. TGF-β1 also induced mRNA for the A chain, but not the B chain, of PDGF. Conversely, PDGF induced TGF-β1 mRNA in sparse but not in dense cultures. These data indicate the existence of a complex inter-dependent regulation of PDGF and TGF-β mRNA expression which is influenced by the cell density.

E 142 EFFECTS OF TRANSFORMING GROWTH FACTOR BETA (TGFß) ON THE PROLIFERATION AND DIFFERENTIATION OF NORMAL AND LEUKEMIC HEMATOPOIETIC PROGENITOR CELLS, Louis M Pelus*, Oliver G Ottmann+, Arnold Ganser+, Dieter Hoelzer+, Depts. of Hematopoietic Regulation*, Sloan Kettering Inst. N.Y., N.Y. 10021 and Hematology+, Univ. of Frankfurt, Frankfurt, FRG.
We investigated the effects of human TGFß1 on the proliferation of normal erythroid (CFU-E,BFU-E), myeloid (CFU-GM) and multipotential (CFU-GEMM) hematopoietic progenitor cells and on leukemic colony forming cells (CFU-c) from 11 patients with Acute Non-Lymphocytic Leukemia (ANLL). In the presence of recombinant hematopoietic colony stimulating factors (CSF), TGFß1 inhibited the proliferation of highly enriched CFU-E and BFU-E (-17 to -73%) and CFU-GEMM (-70 to -98%) over a concentration range of 0.05-1.0 ng/ml. In contrast, CFU-GM stimulated by recombinant Granulocyte-CSF, Granulocyte-Macrophage-CSF or Interleukin-3 (IL3) was enhanced up to 154±22%. The effects of 1 ng/ml TGFß on the proliferation of leukemic CFU-c demonstrated a different pattern of results. In 11 patients with ANLL inhibition by TGFß1 was observed in the range of -67 to +32% (Mean±SD: -41±10%) in the presence of G-CSF, -86 to +1% (-49±8%) in the presence of GM-CSF and -86 to -3% (-47±11%) in the presence of IL3. Enhancement of leukemic CFU-c was observed in only 1 patient. No correlation with FAB classification or % marrow blasts was observed. No difference in the pattern of differentiation or maturational levels of cells in leukemic colonies could be quantitated, and no consistent differences in levels of several maturation antigens was observed by FACS analysis of leukemic blasts after 4 day suspension culture with TGFß. These results indicate that TGFß differentially regulates hematopoiesis and that leukemic CFU-c retain responsiveness to TGF but display a pattern or response similar to normal erythroid and multipotential rather than myeloid progenitor cells.

E 143 ROLE OF C-MYC IN TGFß INHIBITION OF KERATINOCYTE PROLIFERATION, J. A. Pietenpol, C. C. Bascom, H. L. Moses, and J. T. Holt. Dept. of Cell Biology, Vanderbilt Univ. Sch. of Med., Nashville TN 37232. Mouse keratinocytes (BALB/MK), an EGF-dependent epithelial cell line is reversibly growth arrested by pM concentrations of TGFß. TGFß will inhibit EGF-stimulated mitogenesis and decrease c-myc gene expression in rapidly growing and EGF-restimulated quiescent BALB/MK cells. It is proposed that the effect of TGFß on c-myc expression plays a central role in the inhibition of cellular proliferation by TGFß. At one hour following TGFß treatment of rapidly growing BALB/MK cells, c-myc mRNA expression is significantly reduced, and this reduction appears to be controlled at a post transcriptional level as previously evidenced by nuclear run-on analysis. To further analyze the molecular mechanism of TGFß inhibition of c-myc expression, transfection studies were performed. BALB/MK cells transfected with c-myc/CAT promoter constructs and treated with TGFß for 24 hours exhibit a significant reduction in CAT protein production as compared to untreated controls. Together these experiments suggest that there may be two mechanisms of the TGFß reduction of c-myc expression. Initially, c-myc expression is reduced at the post transcriptional level and this is followed by subsequent transcriptional control of gene expression. These data suggest potential autoregulation of c-myc gene expression. Additionally, experiments were designed to investigate whether aberrant regulation of myc results in a loss of responsiveness of the BALB/MKs to TGFß's inhibitory effects. BALB/MK cells have been infected and transfected with v-myc and c-myc respectively. Selected clones are being analyzed to determine whether transformation by v-myc or amplification of c-myc will render the cells insensitive to the growth inhibitory effects of TGFß.

E 144 CHARACTERIZATION OF CELLULAR RESPONSE TO MELANOMA GROWTH STIMULATORY ACTIVITY (MGSA), Ann Richmond, H. Greg Thomas and Rodolfo Bordoni, Department of Medicine, VA Medical Center (Atlanta) and Emory Univ. School of Medicine, Atlanta, GA 30033. Melanoma growth stimulatory activity (MGSA) is an endogenous growth factor for human melanoma cells, cultured nevocytes, and a number of other cell types. MGSA is coded for by a gene with homology to the PDGF inducible competence gene, KC, and the transformation associated human gro gene. In human melanoma cells, the MGSA/KC/gro gene is induced by the MGSA protein, by PDGF, by IGF-1, by cycloheximide, and by TPA. ^{125}I-MGSA binds to a number of cell types and induces a growth response. In some cell types, this binding to the MGSA receptor is accompanied by specific phosphorylations, but the phosphorylated proteins are not precipitable with antibodies to phosphotyrosine. The MGSA response is not mimicked by di-butyryl cAMP (1mM), by isobutylmethylxanthine (0.1mM), or forskolin (50uM). However, when nevus cells were cultured in KGM medium (Clonetics) containing 6ng/ml of MGSA with either 1mM dibutyryl cAMP or 10ng/ml of IGF-1, the growth response was slightly enhanced over that with MGSA alone. Neither di-butyryl cAMP or IGF-1 alone stimulated ^{3}H-thymidine incorporation into DNA in the nevocytes. These data suggest that in nevocytes the MGSA signal transduction pathway does not involve activation of a cAMP dependent pathway.

E 145 EFFECT OF TGF-β_1 ON DNA SYNTHESIS IN NORMAL AND ras-TRANSFORMED NIH3T3 CELLS. Donata Rimoldi, Caroline P. Ford, James L. Cone, Dvorit Samid and Anthony C. Huggett. Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814 and Laboratory of Experimental Carcinogenesis, NCI, NIH, Bethesda, MD 20892.
We have investigated the effects of ras-induced transformation on TGF-β_1 modulation of DNA synthesis in growing and arrested cultures of NIH3T3 cells. We used NIH3T3 cells, v-Ki-ras-transformed NIH3T3 cells (DT), LTR-c-Ha-ras-transformed NIH3T3 cells (RS 485), and nontumorigenic revertants (PR), obtained by treating RS 485 cells with interferon. Both RS 485 and PR cells expressed similar levels of p21 and ras mRNA. Cells were plated at low density in media containing 10% FCS and following attachment TGF-β_1 was added and pulsed ^{3}H-thymidine labelling was performed at various times. TGF-β_1 (0.1 ng/ml) produced a 30-35% inhibition of labelling in NIH3T3 and PR4 cells within 32 h of treatment but little effect was observed in DT and RS 485 cells. In growth-arrested post-confluent cultures of NIH3T3 cells, TGF-β_1 (0.5 ng/ml) produced a stimulation of DNA synthesis which peaked at 36-40 hours following treatment, in contrast to the effect of 10% FCS where stimulation was observed within 24 hours. Preliminary results indicate that under these conditions the PR and RS 485 cells are resistant to the stimulatory effects of TGF-β_1. An analysis of binding of ^{125}I-TGF-β_1 to these cell types indicated no notable differences. These results indicate that TGF-β_1 is a bifunctional regulator of DNA synthesis in NIH3T3 cells and the observed activity is dependent upon the growth state of the cells. In addition, ras-transformed NIH3T3 cells appear to lose their sensitivity to the growth modulatory effects of TGF-β_1 and this cannot be simply explained by a generalized loss of membrane receptors for TGF-β_1. Using this model system it may be possible to delineate the mechanisms involved in the stimulatory and inhibitory effects of TGF-β_1 on DNA synthesis.

E 146 ISOLATION OF NOVEL TGF-β's FROM MEDIUM CONDITIONED BY XENOPUS XTC CELLS. Anita B. Roberts, Frédéric Rosa, Paturu Kondaiah, Martha L. Rebbert, Nanette S. Roche, Igor B. Dawid and Michael B. Sporn. Laboratory of Chemoprevention, National Cancer Institute and Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda, MD 20892.

The observations 1) that medium conditioned by Xenopus XTC cells (XTC-CM) contains TGF-β-like activity; 2) that the ability of XTC-CM to induce formation of mesoderm in explants of frog ectoderm can be blocked by antibodies to TGF-β2, and 3) that TGF-β2, itself, can induce the formation of mesoderm prompted us to attempt to characterize and purify the TGF-β-like activity of XTC-CM. By monitoring inhibition of growth of mink lung CCL 64 cells and utilizing absorption to methyl silyl controlled pore glass and several steps of HPLC on a TSK sizing column and both C18 and CN reversed-phase columns, we have separated 3 distinct TGF-β's each of which migrates characteristically as a 25,000 MW dimer on SDS-PAGE. Although the dose-response curves of inhibition of growth by each of the frog TGF-β's are identical to those of TGF-β's 1 or 2, studies with blocking antibodies specific for TGF-β's 1 and 2 suggest that these frog TGF-β's are different from the known TGF-β's. The mesoderm inducing activity is separated from the TGF-β's by reverse-phase HPLC; none of the frog TGF-β's either alone or in combination is able to induce formation of mesoderm at sub-nanogram concentrations. However, induction of mesoderm is still blocked by antibodies to TGF-β2 suggesting shared epitopes between TGF-β2 and the factor(s) in XTC-CM responsible for induction of mesoderm.

E 147 CHARACTERIZATION OF THE RAF PROTEIN KINASE, David W. Rose, Jan Castagnola, James R. Feramisco, *Gisela Heidecker, and *Ulf R. Rapp, UCSD Cancer Center, San Diego, CA 92103, and *Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, MD 21701

The product of the raf protooncogene is a serine/threonine specific protein kinase which is thought to play a role in the transduction of extracellular signals between the plasma membrane and the cytoplasm. We have used several methods in an attempt to obtain the high level expression of the raf protein in a soluble, biologically active form. We are currently in the process of determining whether raf proteins synthesized in E. coli have similar levels of enzymatic activity as their counterparts in mammalian cells. These proteins are being tested for transformation activity in living cells using needle microinjection. Antibodies directed against the raf proteins are being tested for their ability to inhibit the activity of the enzyme in living cells using this approach as well.

E 148 INHIBITORY EFFECTS OF TGF-β ON IL2- AND IL4-DEPENDENT T CELL PROLIFERATION, J. J. Ruegemer, S. N. Ho, J. A. Augustine, M. P. Bell, J. W. Schlager, D. J. McKean and R. T. Abraham, Mayo Clinic/Foundation, Rochester, MN 55905.

TGF-β inhibits the mitogen-stimulated growth of resting T cells. In the present study, 2 factor-dependent T cell clones were used as defined model systems to examine the mechanism of T cell growth inhibition by TGF-β. The growth of HT-2 cells was stimulated by both IL2 and IL4, whereas CT6 cell growth was strictly IL2-dependent. TGF-β was a potent inhibitor of proliferative responses to both IL2 and IL4. TGF-β treatment blocked, rather than delayed, S-phase entry induced by IL2, but was most effective if added within 4 hours of growth factor stimulation. In HT-2 cells, a 3 hr TGF-β treatment decreased high affinity IL2 receptor expression by 30-40%, without altering the ligand binding affinity. In contrast, high affinity IL2 receptor expression on CT6 cells was not altered by TGF-β. In both cell lines, TGF-β inhibited the IL2-dependent increase in transferrin receptor expression by 50-60%. IL2 or IL4 stimulation of G_1 phase-arrested T cells resulted in the early expression of several growth-related and T cell-specific genes. TGF-β treatment selectively blocked IL2- and IL4-dependent increases in c-myc and GM-CSF gene expression in HT-2 cells, and c-myc gene expression in CT6 cells. The inhibitory effects of TGF-β on growth factor-mediated mRNA accumulation in the T cells was due to interference with the transcriptional activation of specific genes by the growth factors. These studies indicate that TGF-β inhibits T cell proliferative signals generated by both IL2 and IL4 receptors, and suggest that growth factor-stimulated c-myc gene expression may represent a critical intracellular target for the anti-proliferative effects of TGF-β on T cells.

E 149 IN VIVO ADMINISTRATION OF TRANSFORMING GROWTH FACTOR β1 SELECTIVELY INHIBITS MURINE HEMATOPOIETIC PROGENITOR CELL PROLIFERATION. F.W. Ruscetti1, H. Goey2, J.R. Keller2, T. Back2 and R.H. Wiltrout3. 1Lab. of Mol. Immunoreg., 2BCDP-Program Resources, Inc., and 3Lab. Exp. Immunol. NCI-Frederick Cancer Research Facility, Frederick, MD 21701 Transforming growth factor β (TGF β) is 25 Kd homodimeric protein with both growth-enhancing and inhibiting properties. In previous studies, we have shown that TGF β1 and TGF β2 are potent selective inhibitors of murine and human hematpoiesis. Early hemato-poeitic cells such as high proliferative potential colony forming cells, CFU_{GEMM}, which gives rise to colonies consisting of granulocytes, erythroid, macrophages, megakaryocytes, the erythroid burst forming cell (BFU_E) and CFU_{GM} which gives rise to granulocytes and monocytes were inhibited by TGF β1 but CFU_G, CFU_M, CFU_E, the differentiated colony forming cells which give rise only to granulocyte, macrophage and erythroid cells respectively were not inhibited. The suggestion that TGF β is a negative regulator of hematopoietic cell growth was tested in vivo. A surgical technique was developed to administer TGF β1 loco-regionally via injection in the femoral artery. Relatively small amounts (1 ug/mouse) of TGF β1 inhibited the baseline and interleukin-3 driven proliferation of bone marrow cells in a time and dose dependent manner. Hematopoietic colony formation as assayed by CFU_{GEMM}, BFU_E and CFU_{GM} formation was inhibited by TGF β1 in vivo. The single lineage colonies, CFU_G and CFU_M were not affected. Thus, the effects of TGF β1 on in vitro hemato-poiesis can also be observed after in vivo administration of TGF β1 suggesting that TGF β is a physiologically relevant regulator of hematopoiesis. Thus, TGF β1 may be useful in protecting progenitor cells from the myelotoxicity of chemotherapeutic drugs.

E 150 MODULATION OF TYPE IV COLLAGENASE GENE EXPRESSION IN HUMAN SKIN FIBROBLASTS BY TPA, TGF-β AND EGF-1, Tuula Salo, Pirkko Huhtala and Kari Tryggvason, Biocenter and Department of Biochemistry, University of Oulu, SF-90570 Oulu, Finland. Type IV collagenase is a neutral metalloproteinase that specifically degrades type IV collagen, the major structural component of basement membranes. Type IV collagenase is secreted in low amounts by proliferating fibroblasts but in high quantities by metastatic tumor cells. In the present work we have studied the modulation of type IV collagenase gene expression in skin fibroblasts by TPA, TGF-β and EGF-1. TPA (10^{-8}M) and TGF-β (2ng/ml) caused an about three-fold increase both in the levels of enzyme activity and mRNA. With TPA the increase in the level of type IV collagenase mRNA became visible after 5 hours whereas with TGF-β only after 11 hours. EGF-1 had no significant effect. The results differ from those observed in human fibrosarcoma cells (HT-1080) that secrete normally high amounts of type IV collagenase. In those cells TPA caused no increase in gene transcription whereas TGF-β did. These results indicate that TPA and TGF-β induce gene expression by different mechanisms.

E 151 TRANSFORMATION OF NORMAL MOUSE MAMMARY EPITHELIAL CELLS FOLLOWING TRANSFECTION WITH A HUMAN TRANSFORMING GROWTH FACTOR ALPHA cDNA, Fortunato Ciardiello, Vidya Shankar, Nancy Kim, Rik Derynck, Nancy Hynes, Marc Lippman, and David S. Salomon. Laboratory of Tumor Immunology and Biology and Medicine Branch, NCI, NIH, Bethesda MD; Genentech Inc., South San Francisco, CA and Ludwig Institute for Cancer Research, Bern, Switzerland. To determine whether enhanced expression of transforming growth factor α(TGFα) is sufficient to induce neoplastic transformation of normal mammary epithelial cells, we cotransfected NOG-8 cells, a normal cloned mouse mammary epithelial cell line, with an SV40-TGFα expression vector and with pSV2neo. Following cotransfection, 9 G418 resistant NOG-8 colonies were cloned and expanded. All clones were subsequently analyzed for TGFα production, for anchorage-independent growth in soft-agar, for EGF receptor ex-pression and for tumorigenicity in nude mice. Among the 9 NOG-8 transfected clones, 3 clones secreted high levels of TGFα into the culture medium (177 to 600 ng/10^8 cells/48 hrs) and grew aggressively in soft agar. NOG-8 clones selected from cells transfected with the pSV2neo plasmid alone failed to grow in soft agar and secreted low levels of TGF α(7 ng/10^8 hrs). There was a significant inverse relationship between the number of EGF recep-tors expressed on the transfected NOG-8 clones and the amount of biologically active TGFα secreted by these cells. In addition, 3 clones of NOG-8 cells that secreted high levels of TGFα and that were able to grow in soft agar and formed undifferentiated carcinomas in nude mice. These results suggest that overexpression of the TGFα gene in normal mouse mammary epithelial cells can induce anchorage-independent growth in vitro and can lead to a tumorigenic phenotype in vivo.

E 152 STIMULATION OF PRIMARY NON-SMALL CELL LUNG TUMOR CELLS BY AUTOCRINE GROWTH FACTORS, Jill M. Siegfried, Department of Pharmacology, University of Pittsburgh, Pittsburgh, PA 15261
Primary non-small cell lung tumor cells were used to assess the growth-promoting activity of conditioned medium (CM) from A549 cells. CM stimulated colony formation in monolayer culture and in an anchorage-independent growth assay. The extent of response varied from 1.7 to 6.6-fold over control in the monolayer assay, using cells derived from different tumors. In the anchorage-independent growth assay, the variation in response was 2.0 to 7.9-fold. CM added to culture medium enhanced the success rate of establishing short-term and long-term cell cultures from primary solid tumors. Of 22 cases placed in culture, primary cultures were obtained in 20 cases when cultured with CM. Subcultures were obtained in 18 cases, and 9 cell lines were established. These cultures were proven to be of malignant origin by their altered expression of lactate dehydrogenase isoenzymes, expression of pregnancy-specific ß-glycoprotein, and formation of carcinomas in immuno-compromised mice. Normal bronchial epithelial cells were used as controls in these assays. CM was shown to contain both Transforming Growth Factor α (TGFα) and Insulin-like Growth Factor-1. The combination of both these growth facotrs added as purified peptides at levels found in CM (0.1-0.5 ng/ml) stimulated the colony formation of primary lung tumor cells in monolayer culture by 5-fold.

E 153 IDENTIFICATION OF cDNA CLONES WITH ALTERED REGULATION IN TGFß-RESISTANT K-ras TRANSFORMED MURINE KERATINOCYTES. Nancy J. Sipes, Duncan A. Miller, Lynn M. Matrisian, and Harold L. Moses. Dept. of Cell Biology, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232 Growth factor regulation of normal epithelial cells involves negative and positive signals. We have characterized such requirements in mouse keratinocytes (BALB/MK cells). TGFα is a strong mitogen for these cells, while TGFß is a potent inhibitor of cellular proliferation. Transformation by activated K-ras is sufficient to abrogate the EGF/TGFα requirement, yet these derivatives (KC cells) still maintain their growth arrest response to TGFß. We have isolated variants of the KC cells which no longer respond to concentrations equal to and greater than those that inhibit growth of the nonselected parental cells. These TGFß-resistant cells (KCR) have been characterized as to TGFß receptor content and growth-associated gene expression. As the KCR cells exhibit TGFß receptors and show similar actions on expression of c-myc, c-gro, TGFα, TGFß, and K-ras after TGFß treatment, we conclude that the mechanism of nonresponsiveness is distal to receptor-ligand interactions and early signal transduction pathways. We chose a molecular cloning strategy to further evaluate this resistance. A cDNA library was constructed from KCR mRNA (STRATAGENE, Inc.) and screened with a cDNA probe generated from KCR cells treated with TGFß for 1 hour and subtracted with excess mRNA from TGFß-treated KC cells. Independent clones were identified after three screenings, of which two clones (ßR-2 and ßR-3) were selected for further characterization. Northern analysis revealed TGFß inhibition of ßR-2 and ßR-3 mRNA levels in the sensitive KC cells but not in the resistant KCR cells. These clones represent genes that are potentially important to TGFß action, and may provide information regarding the mechanism of TGFß nonresponsiveness in neoplastic progression.

E 154 EFFECTS OF TRANSFORMING GROWTH FACTOR-ß ON HUMAN MAMMARY EPITHELIAL CELLS IN CULTURE, Martha Stampfer, Myriam Alhadeff, Dennis Prosen, Mina Bissell, and Junko Hosoda, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.
The growth of normal human mammary epithelial cells (HMEC) in serum-free medium in culture is inhibited by TGF-ß. The extent of growth inhibition varies among individual specimen donors and as a function of age in culture. Two HMEC cell lines, derived from normal cells transformed to immortality following *in vitro* exposure to benzo(a)pyrene, have varying growth responses to TGF-β. Uncloned populations of line 184B5 are largely insensitive to TGF-ß induced growth inhibiton, while uncloned 184A1 is largely TGF-β growth sensitive. However, clonally isolated populations of both lines may be either sensitive or insensitive. Proliferating normal and transformed HMEC express c-k-ras, c-myc, c-fos, and c-erbB-2 in culture. Expression of c-fos and c-myc is reduced in TGF-β exposed normal cells. We are currently evaluating expression of these genes in the TGF-β exposed sensitive and insensitive immortalized cell lines. Normal HMEC and 184B5 show a large increase in protein synthesis and secretion per cell in response to TGF-β. mRNA and protein levels for fibronectin and collagen IV are increased 4-8 fold in both cell types, although the normal HMEC are growth inhibited while the 184B5 are not. The levels of many other, as yet unidentified secreted proteins, are also increased in both cell types in response to TGF-β. Thus epithelial cells, like mesenchymal cell types, may be induced for extracellular matrix prtoein synthesis by TGF-β; this induction is independent of the effect of TGF-β on cell growth.

E 155 COMPARISON OF URINARY TRANSFORMING GROWTH FACTOR-ALPHA (TGF-ALPHA) IN PATIENTS WITH DISSEMINATED BREAST CANCER AND HEALTHY CONTROL WOMEN. Kurt Stromberg[1,2], Maribeth Duffy[2], Christina Fritscn[2], Susan E. Bates[3], and W. Robert Hudgins[2]. Division of Cytokine Biology, FDA, Bethesda, MD, 20892[1]. Uniformed Services University, Bethesda, MD 20814-4799[2], and National Cancer Institute, Bethesda, MD, 20892[3].
In an effort to explore polypeptide growth factors as potential markers for cancer detection, we have identified the presence of TGF-alpha in pooled urine of disseminated breast cancer patients by a commercial radioimmunoassay (RIA) based on a rabbit antiserum raised to the C-terminal 17aa synthetic fragment of rat TGF-alpha (Biochem. Biophys. Res. Commun. 144:1059-1068, 1987). This TGF-alpha RIA detected low molecular weight (LMW) 6 kDa bioactive synthetic rat and human TGF-alpha equally well, both high molecular weight (20-30 kDa by Bio-gel P-100 chromatography) and fully processed LMW urinary human TGF-alpha, and did not cross react with either HMW or LMW human urinary EGF. Using established procedures (Cancer Res. 46:6004-6010, 1986) of concentrating urinary proteins from 24 hr urine samples by absorption onto methyl bonded microparticulate silica, and selective elution by acetonitrile, scattergram tabulation of TGF-alpha RIA results were compared from women with disseminated breast carcinoma and healthy pre- and post-menopausal control women. Statistical analysis indicated a mean TGF-alpha value of 1013 ng/gm urinary creatinine for tumored urine samples (range 604 to 1810) and 612 ng/gm creatinine (range 430 to 960) for control urine samples. With this assay method, urinary TGF-alpha detection appears to have limited usefulness as a diagnostic marker for metastatic human adenocarcinoma of breast.

E 156 A RAS ONCOGENE SUPPRESSOR FUNCTIONS BY INHIBITING SIGNAL TRANSDUCTION THROUGH THE EPIDERMAL GROWTH FACTOR RECEPTOR, Michael A. Tainsky, Paul Chiao, Sun O.Yim and David Krizman, Dept. of Tumor Biology, University of Texas, M.D. Anderson Hospital Cancer Center, Houston, Texas

A human cell culture system has been developed to identify how oncogenes' action is regulated during the of transitions in multistage carcinogenesis. PA-1 human teratocarcinoma cells show progression as they are passaged in culture. Certain preneoplastic cells in this PA-1 series contain genes which make them susceptible to transformation by single oncogenes while others require both myc and ras oncogenes to induce tumorigenesis. The molecular genetic basis of this susceptibility to single oncogene induced transformation appears to be due to loss of a suppressor gene. Myc must therefore by-pass the regulatory effects of the suppressor gene. We have found that the mechanism by which cells acquire the susceptibility to ras is related to responsiveness to epidermal growth factor and transforming growth factor-alpha possibly by affecting an autocrine mechanism. This represents a new concept for inactivation of a suppressor gene during a preneoplastic stage progressing toward tumorigenicity. We will discuss differences in the expression oncogenes and growth factors as well as other transformation sensitive genes in cell lines in the PA-1 series representing various stages of tumor progression with regard to loss of a suppressor gene.

E 157 EFFECTS OF TGFβ ON THE PROLIFERATION, CHEMOTAXIS AND BASEMENT MEMBRANE INVASIVENESS OF CELL LINES WHICH MODEL DIFFERENT STAGES OF HUMAN BREAST CANCER. Erik W. Thompson[1], Thomas B. Shima[1], George R. Martin[1], Eva M. Valverius[2], Marc E. Lippman[2] and Robert B. Dickson[2]. [1]LDRA, NIDR, NIH, Bethesda, MD 20892, and [2]Lombardi Cancer Center, 3800 Reservoir Rd., NW Washington DC 20007.
We examined the effects of TGF-β on basement membrane-invasiveness of cell lines which model the malignant progression from normal mammary epithelium through estrogen - dependent to estrogen - independent breast cancer. We used immortalized human mammary epithelial cells (184A1N4) transformed with the viral oncogenes v-Ha-ras (A1N4-H), SV40-T antigen (A1N4-T), or both (A1N4-TH; Cancer Res. 48:4689, 1988), which show a stepwise progression to a fully malignant phenotype. We have also examined the estrogen-dependent MCF-7 and estrogen-independent MDA-MB-231 cell lines. Treatment of monolayer cultures with TGF-β (5ng/mL) inhibited cellular proliferation by 40-80%, with the least effect seen on the estrogen independent MDA-MB-231 and fully malignant A1N4-TH cells. The ability to penetrate reconstituted basement membrane (Matrigel) in the chemoinvasion assay (Cancer Res. 47:3239,1987) was highest in the more progressed cell lines: MDA-MB-231 = A1N4-TH > A1N4-H = A1N4-T > MCF-7 > 184A1N4. TGF-β stimulated the invasiveness of the 184A1N4 and MCF-7 cell lines, had no effect on the A1N4-T or A1N4-H lines, but inhibited the invasion of the A1N4-TH and MDA-MB-231 cells. Chemotaxis to fibroblast conditioned media, the same chemoattractant used in the chemoinvasion assay, was partially inhibited in all cases. The data suggest that TGF-β has differential effects on human breast cancer cell invasiveness, stimulating less malignant and hormone - dependent cell lines, and inhibiting those which have progressed to an estrogen-independent, more malignant and invasive phenotype. These differential effects on invasiveness contrast the universal inhibition of chemotaxis and proliferation caused by TGF-β in these cells, but are consistent with the known pleomorphism displayed by TGF-β in it's effects on various cellular parameters.

E 158 TRANSFORMING GENES IN AML, Deniz Toksoz and Christopher J. Marshall, Institute of Cancer Research, Chester Beatty Laboratories, London SW3 6JB, U.K.

We are studying transforming genes in acute myeloid leukemia patients using the NIH 3T3 transfection assay followed by tumor formation in nude mice. Previous results have shown that while an activated ras gene is detected in 30% of AMLs, the cells containing mutant ras are frequently a minor subclone within the total leukemic population (Toksoz, Farr & Marshall, Oncogene 1; 409, 1987). We have now studied 3 cases of presentation-relapse sets where an activated N-ras occurs in the presentation DNA. Using the in vivo tumor bioassay we detected an activated N-ras gene in a transfection from only one of the relapse samples. Furthermore, the polymerase chain reaction (PCR) was used to show that in this case the mutation is not the same base substitution as found in the presentation DNA and occurs only in a minor fraction of the total blast population. These results indicate that ras gene activation may not be an initial leukemogenic event in AML, and suggest that both presentation and relapse phases of the disease may involve the activation of other oncogenes in addition to ras. For this reason we have isolated non-ras transforming activity from leukemic patient DNA using the tumor bioassay. Three DNA samples have been found to contain human ALU repeats in the second round of transfection. One of these (CR) has yielded tumors in a third round of transfection with a conserved ALU fragment pattern of 5 EcoRI bands which show no hybridisation on Southern blots with N-, H-, Ki-ras, c-raf-1, hst, fms, abl, c-erb-b-2, mas and trk. In order to clone this gene a lambda library was made from the tertiary tumor and ALU repeat-containing clones were isolated. Results of further characterisation of these clones will be presented.

E 159 Activated Raf Gene Alters Growth Characteristics of Interleukin-2(IL-2) Dependent T-Cells, Bruce C. Turner, Gisela Hiedecker*, Ulf Rapp*, John C. Reed, Dept. of Path., University of Pennsylvania, Philadelphia, Pa. and * Lab. of Viral Carcinogenesis, NCI, Frederick, MD. The c-raf proto-oncogene is the cellular homologue of the transforming gene of the MSV 3611 retrovirus. This gene encodes a cytosolic kinase with specificity for serine and threonine residues. Activation of the c-raf kinase can be achieved by removal of its NH2-terminal regulatory domain. Because c-raf is expressed in normal T-cells and because IL-2 induces rapid phosphorylation of proteins in these cells, we wondered whether raf might participate in IL-2 mediated responses. We stably introduced normal and activated forms of the human c-raf gene into the IL-2 dependent T-cell clone CTLL-15H by retrovirus-mediated gene transfer. Retroviral constructs containing a neomycin-resistance gene and either normal or activated c-raf were packaged as amphotrophic virus and used to infect T-cells. After selection in neomycin, in the continued presence of IL-2, stably infected cells were assayed with regard to their growth characteristics. These experiments demonstrated: 1) that activated raf (but not normal c-raf) increased the proliferative rate by 5-10 fold at all concentrations of IL-2 and 2) allowed T-cells to survive longer in culture in the absence of IL-2, requiring several days to reach quiescence. Responsivity to IL-2 however was not significantly different for cells infected with normal c-raf or activated c-raf. These experiments represent a first step in elucidating the role of the raf gene in interleukin-induced proliferation of T lymphocytes.

E 160 HUMAN GENOMIC SEQUENCES WHICH SUBSTITUTE FOR THE IMMORTALISING FUNCTION OF SV40 LARGE T ANTIGEN. Deon J Venter, Timothy E W Riley, Zebunnisa Ikram, Karen H Vousden, Mark D Noble and Parmjit S Jat. Ludwig Institute for Cancer Research, London, UK.

We have used a conditionally immortal cell line derived by infecting rat embryo fibroblasts with the temperature-sensitive (ts)large T antigen from SV40tsA58, to look for novel human immortalising genes (1). At the permissive temperature these cells divide indefinitely whilst at the non-permissive temperature,at which large T is degraded, they undergo rapid growth arrest. We have shown that this growth defect is readily overcome by the introduction of exogenous wild-type SV40 large T antigen (T), Adenovirus 5 E1A, (1), Polyoma Early Region, and HPV16 E7 gene products, (2), but not by the introduction of the c-*myc* and v-*myc* genes. Since recent work has shown that the E1A, E7, Polyoma large T (3), and SV40 T proteins (4) bind to the retinoblastoma gene product (RB), it is possible that the mechanism of immortalisation of this cell line may be linked to the binding of RB to T at the permissive temperature. The mechanism of rescue of the growth defect by the wild-type viral oncogenes may similarly be due to binding of RB.This cell line may thus help identify human genes which would act in a manner analogous to wild-type T, E1A, and E7.

We have successfully overcome the ts growth defect of these cells by transfecting into them high molecular weight human DNA derived from two sources: 1)a malignant glioma,and 2) embryonic lung fibroblasts. These human DNA sequences appear to substitute for the immortalising function of degraded ts T, and a second round of transfection is underway in order to characterise them further.

1)Jat PS and Sharp PA "Cell lines established by a temperature-sensitive SV40 large T antigen gene are growth restricted at the non -permissive temperature. (1988, submitted.) 2)Vousden KH and Jat PS "Functional similarity between HPV16 E7, SV40 large T and Adenovirus E1A proteins. (1988, submitted.) 3)Whyte P, et al. (1988) Nature 334, 124-129. 4)DeCaprio J, et al (1988) Cell 54, 275-28

E 161 RECOMBINANT TGF-β1 IS SYNTHESIZED AS A TWO-COMPONENT LATENT COMPLEX THAT SHARES SOME STRUCTURAL FEATURES WITH THE NATIVE PLATELET LATENT TGF-β1 COMPLEX, Lalage Wakefield, Diane Smith, Susan Broz*, Mark Jackson*, Arthur Levinson* and Michael Sporn; Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892 and *Dept. of Molecular Biology, Genentech Inc., So. San Francisco, CA 94080. The entire coding region of the human transforming growth factor-β1 (TGF-β1) precursor cDNA has been expressed in a human renal carcinoma cell line. Like platelet TGF-β1, the recombinant TGF-β1 is secreted in a biologically latent form. Immunoblot analysis and gel filtration indicate that the recombinant latent TGF-β1 is a 100 KDa complex in which active 25 KDa TGF-β is non-covalently associated with the remaining 75 KDa of the processed precursor. Unlike the platelet latent complex, the recombinant latent complex contains no 135 KDa component. Thus the processed precursor peptide alone is sufficient to confer latency on active TGF-β1, and the 135 KDa platelet component has a different role. The processed precursor is similarly glycosylated in recombinant and platelet complexes, and in both has an exposed heparin binding site that may be involved in targeting of the latent complex. Activation of recombinant and platelet complexes is reversible, suggesting that the activation process does not cause any major structural modifications in the components of the latent complex. The structural differences between the recombinant and native latent forms should yield information about the mechanics of regulation of TGF-β through activation of the latent form, and may be exploitable therapeutically.

E 162 EFFECTS OF TRANSFORMING GROWTH FACTOR BETA AND EPIDERMAL GROWTH FACTOR: SYNERGISM ON DNA SYNTHESIS WHICH IS UNCOUPLED FROM EARLY GENE EXPRESSION, Charles E. Wenner, Ann M. Neilson, Jennifer Brecht and Anthony F. Cutry, Department of Experimental Biology, Roswell Park Memorial Institute, Buffalo, NY 14263.
We have investigated the effects of transforming growth factor beta (TGFβ) and epidermal growth factor (EGF) on DNA synthesis and early gene expression in C3H 10T1/2 fibroblasts. Previous reports had indicated that the combination of TGFβ and EGF was capable of causing morphological transformation of some cell types, indicated by growth in semi-solid medium. This phenotype was reversible upon removal of the growth factors from the culture medium. Therefore, we sought to investigate the effect of these two growth factors on DNA synthesis. We find that EGF alone is capable of inducing a significant increase in radiolabelled thymidine incorporation, and that TGFβ is able to markedly potentiate this response, indicative of a strong synergistic effect of the two mitogens on DNA synthesis. TGFβ alone was a comparatively weak inducer of DNA synthesis. Since stimulation of the c-fos and c-myc protooncogenes is almost always observed upon activation of cell-cycle progression, we then studied whether the synergistic effect of EGF and TGFβ could be due, in part, to synergism with respect to induction of c-fos and c-myc mRNAs. We have previously described the induction of these mRNAs by EGF and TGFα in 10T1/2 cells, and find that TGFβ alone is capable of inducing a small (relative to EGF) transient increase in c-fos mRNA levels, but has no effect on c-myc mRNA levels. The combination of EGF and TGFβ revealed no synergistic effect on the expression of either c-fos or cmyc mRNAs. Therefore, it appears that the synergistic effect of the two ligands on DNA synthesis is unrelated to their effects on early gene expression. We are exploring the possibility that TGFβ and EGF may synergize with regard to the induction of gene expression at later time points in the cell cycle.

E 163 PROTEIN KINASE C DIFFERENTIALLY INHIBITS EPIDERMAL GROWTH FACTOR RECEPTOR OPERATED CALCIUM ENTRY IN A431 CELLS, Larry A. Wheeler, Danon Goodrum and George Sachs, Dept. of Biological Sciences, Allergan/Herbert Labs Inc., Irvine, CA 92715
Activation of the epidermal growth factor receptor (EGFR) by either epidermal growth factor (EGF) or transforming growth factor-alpha (TGF-α) stimulates a rise in free cytosolic calcium ($[Ca^{2+}]_i$) that involves transmembrane Ca^{2+} entry, release of Ca^{2+} from intracellular stores and activation of Ca^{2+} pump for Ca^{2+} extrusion. A431 cells were loaded with fura-2 to examine the effects of protein kinase C (PKC) activation on these different modes of regulating $[Ca^{2+}]_i$. Cells treated with $5x10^{-8}$M 12-O-tetradecanoyl-phorbol-13-acetate (TPA) inhibited both EGF-induced Ca^{2+} entry and Ca^{2+} release whereas $5x10^{-10}$M TPA for 2 min had no effect on the initial Ca^{2+} transient that peaked in 15-30 sec after addition of EGF or TGF-α but stimulated a rapid return of $[Ca^{2+}]_i$ to baseline. Addition of 3-10μM R 59022, a diglyceride kinase inhibitor secondarily had a similar effect on the EGF-induced rise in $[Ca^{2+}]_i$. Staurosporine (1-10nM) completely inhibited the effects of TPA supporting the concept of PKC activation inhibiting Ca^{2+} entry. To distinguish between the possibility that TPA was activating Ca^{2+} pumps rather than inhibiting Ca^{2+} entry fura-2 loaded cells were suspended in buffers containing Mn^{2+}. Addition of EGF or TGF-α induced Ca^{2+} release and subsequent Mn^{2+} influx. TPA (10^{-10}M) blocked Mn^{2+} influx but not the inactive phorbol ester 4α-phorbol-12, 13-didecanoate. These results suggest that activation of PKC plays a role in turning off EGF activated Ca^{2+} entry. This concept was tested with downregulation of PKC by incubating cells 24 hrs with 1μM TPA. Addition of EGF resulted in prolonged Ca^{2+} entry supporting a role for PKC in EGFR-induced Ca^{2+} entry.

E 164 DOWN-REGULATION OF PROTEIN EXPRESSION IN v-raf AND v-raf/v-myc TRANSFORMED RAT LIVER EPITHELIAL CELLS, Peter J. Worland, Lori L. Hampton, Anthony C. Huggett and Snorri S. Thorgeirsson, Laboratory of Experimental Carcinogenesis, NCI, NIH, Bethesda, MD 20892. Rat liver epithelial (RLE) cells were infected with a defective retrovirus containing either v-raf (R3611) or a combination of v-raf/v-myc (RJ2) as well as the helper virus (RLEC). Single cell clones RLEC-2, RJ2-14, R3611-3 and R3611T-2 were derived and they exhibited quite distinct cell morphologies from the regular cuboidal shape typical of RLE cells, to elongated spindle shapes and cells growing in suspension. All v-raf infected cells expressed comparable levels of v-raf mRNA. The RJ2-14 cells were able to grow in soft agar, while the other clones could not. All clones except the RLEC-2 cells formed tumors in nude mice, but with different latency periods (RJ2-14, R3611T-2 latency = 2 days, R3611-3 latency = 26 days) and different growth rates. Two proteins of pI/M_r 5.9-7.2/205 and 6.5-7.5/160 indicated by 2-dimensional polyacrylamide gel electrophoresis were expressed in the RLEC-2 and R3611-3 cells but absent from the RJ2-14 and R3611T-2 cells. Repeated gels run from cell samples taken over a period of weeks in culture determined this finding to be consistent. Crude fractionation studies determined the 205K protein to be soluble and the 160K protein to be located in the membrane. This membrane bound protein contains mannose and galactose residues in its structure. The R3611-3 clone expresses both of these proteins in culture, but they were absent in tumors generated from these cells in nude mice. Therefore, the expression of these proteins appear to be down-regulated by the process of tumor formation and not the expression of v-raf.

E 165 A 3.1 KB DNA SEQUENCE, hhc^M, ISOLATED FROM MAHLAVU HEPATOCELLULAR CARCINOMA CELLS, TRANSFORMS RAT LIVER CELLS BESIDES NIH3T3 CELLS, AND ALSO CODES FOR A 57,000 DALTON PROTEIN, Stringner S. Yang[1], K. Zhang[1], J. Taub[1], W. Vass[1], and W. Vieira[2]. [1]LCO and [2]LCB, NCI, Bethesda, MD 20892.

A 3.1 kb human genomic DNA sequence, hhc^M, isolated from an African hepatocellular carcinoma (HHC Mahlavu) cell line, transformed NIH3T3 at a low efficiency. hhc^M was placed in a neomycin resistance-SV40 promoter vector (pN^r; 5.8 kb). One clone, designated pN^rpM-1, transformed buffalo rat liver (BRL-1) cells as well as NIH3T3 cells and passed the human DNA sequence stably in secondary and tertiary transfections. BRL-1 cells appear contact-inhibited and are non-tumorigenic. When transfected with pN^rpM1 DNA, BRL-1 cells developed neomycin-resistance ($G418^r$) and grew in Gentamicin sulfate. $G418^r$ colonies in both transformed NIH3T3 and BRL-1 cultures also showed anchorage independent growth when screened by colony formation in soft agar. Soft agar clones, especially NIH3T3 transformants, were highly tumorigenic in Swiss nu/nu mice and athymic nu/nu rats. $G418^r$ BRL-1 and NIH3T3 cells transfected with pN^r DNA formed occasionally 1 or 2 tiny colony; but they never developed tumors in athymic Swiss nu/nu mice. The transformants showed hhc^M DNA by Southern-blot hybridization. Secondary transfection with the purified transformant DNAs also succeeded in the transfers of the hhc^M DNA sequence, $G418^r$ phenotype and tumorigenicity to the secondary transfectants. Messenger RNAs, Prepared from hhc^M transformed NIH3T3 tumor cells and Mahlavu HHC cells, and selected by hybridization against hhc^M DNA, coded for a 57,000 dalton protein in cell-free protein synthesis using the rabbit reticulocyte lysate system, corroborating with the sequencing data of hhc^M, that it contains an ORF.

E 166 Multiple Proteins interact with the Gibbon Ape Leukemia Virus (GALV) Enhancer element. A. Rosella Farina, John P. Quinn and David L. Levens. Lab. of Pathology, NCI, NIH. Bethesda MD. We have previously determined that the major determinant of enhancer activity in the Gibbon Ape Leukemia Virus (GALV) residues within a 22 bp sequence. This element is transactiveted by a complex of proteins. This element contains a consensus sequence for the AP1 protein binding site which interacts with cjun and cfos in Hela cells. However it would appear that different factors interact with this sequence in MLA144 cells, a cells line which has high enhancer activity. We have studied the mechanism of transactivation in MLA 144 cells and attempted to purify the proteins from this cell line which transactivate this element. Using copper footprinting and contact point analysis we have studied the interaction of these proteins, separately or as a complex with this element. We have compared this analysis with another known enhancer element which also contains an AP1 site; and determined that different protein interact with these AP1 site.

Tyrosine Kinase- Related Oncogenes; Repression

E 200 PHOSPHORYLATION OF MYELIN ASSOCIATED GLYCOPROTEIN ON SERINE, THREONINE AND TYROSINE RESIDUES, Aled Edwards, Daniel Afar, Peter Braun and John C. Bell, Department of Biochemistry, McGill University, Montreal, Quebec, Canada, H3G 1Y6

Myelin associated glycoprotein (MAG) is a transmembrane molecule thought to be involved in the process of molecular recognition and signal transduction between cells. MAG exists as at least two isoforms as the result of developmentally regulated alternative splicing of the same primary transcript. The larger form (L-MAG) contains at its carboxy terminus a domain which is highly homologous to the major autophophorylation site of the EGF receptor as well as a potential protein kinase C site. Recently, we have shown by direct injection of 32P into mouse brains that L-MAG can be phosphorylated on serine, threonine and tyrosine residues. The other isoform of MAG (S-MAG) lacks this potential phosphorylation domain and is not phosphorylated in vivo or in vitro. To facilitate both biochemical and biological studies of the two MAG isoforms, we have cloned the corresponding cDNAs into retroviral expression vectors and have introduced the genes into NIH 3T3 cells. Our results indicate that the phosphorylation pattern observed on MAG molecules expressed in the brain is conserved in these cell lines and can be manipulated by a variety of pharmacological agents. We are attempting to establish additional cell lines which we can use in adhesion assays to measure the biological properties of MAG. Ultimately we will use site directed mutagenesis to determine the role phosphorylation plays in regulating MAG.

E 201 THE MAJOR TYROSINE PROTEIN KINASE ACTIVITY PURIFIED FROM HL-60 IS NOT SUPPORTED BY $PP60^{SRC}$ OR ANY RELATED PROTEIN SPECIES, Jean A. Boutin, Anne-P. Ernould and Claude A. Cudennec, Division de Pathologies Cancereuses, Institut de Recherches Servier, 11 rue des Moulineaux - 92150 Suresnes-France

A major peak of tyrosine protein kinase activity (TPK) is eluted when the 0.1 % Triton X100 extract from HL-60 homogenate is subjected to a DEAE-sepharose and then to a Cibacron blue agarose chromatography. At least, three other minor peaks of activity were left over during the course of the experiments. This preparation was insensitive to EGF and insulin. This preparation submitted to HPLC gel filtration eluted at a volume corresponding to a mass of 35/38 kD. Further purification steps comprising a tyrosine-agarose and a phenyl-sepharose led to a highly purified preparation. Autoradiographs of the preparations incubated with labelled ATP and separated by electrophoresis do not give any evidence that autophosphorylation does occur for that particular TPK. Furthermore, it was impossible, in our hands to immunoprecipitate the enzyme with a specific antiphosphotyrosine antibody. Similarly, there was no evidence that the activity could be immunoprecipitated with anti-src antibody. A preliminary set of enzymological experiments is presented which characterizes the specificity and inhibitions of this TPK activity. All the data presented herein suggest that the enzyme purified in our laboratory has not been previously described in the litterature.

E 202 ACTIVATION OF THE TRANSFORMING ACTIVITY OF THE HUMAN *NEU* GENE BY A POINT MUTATION IN THE TRANSMEMBRANE DOMAIN. Bruskin, A., Johnson, K., Fay, R., Panicali, D. and Andrea, N. Oncogenetics Partners, Cambridge, MA 02142

Activation of the rat *neu* oncogene has been shown to be caused by the replacement of valine 664 with a glutamic acid. Oligonucleotide directed mutagenesis was used to introduce a glutamic acid codon at the homologous position in the human *neu* allele (aa #659). Introduction of this *neu* allele, by infection with a retrovirus expressing *neu* and neo^R under independent promoters, into NIH 3T3, Rat-1, or C57A cells (a normal murine mammary cell line) results in the transformation of these cells. The unmutated *neu* allele does not transform these cells when expressed in the same vector. When the mutated *neu* allele was introduced into human cell lines, HOS and HBL-100 (normal human mammary cell line) the infectivity was down 25 fold and all $G418^R$ cell lines were not transformed. These data suggest that the expression of the mutated human *neu* allele is toxic to nontransformed humans cells. This toxicity seems to be overcome in transformed cells. Infection of MDA-MB-436 cells, a human breast tumor cell line, by the virus expressing the mutated *neu* yields cell lines that express the mutated *neu* gene. To further investigate the toxicity of the mutated human *neu* gene, plasmids will be constructed that express the mutated *neu* gene under the control of the MMTV LTR. Cell lines with integrated copies of the MMTV-*neu* will be tested for growth in the presence and absence of dexamethasone.

E 203 IDENTIFICATION AND CHARACTERIZATION OF THE SITES OF TYROSINE PHOSPHORYLATION AT THE AMINO AND CARBOXY TERMINI OF pp60$^{c\text{-}src}$. *Seng H. Cheng, Robert Harvey, Pearl C. Espino and Alan E. Smith, Laboratory of Cellular Regulation, Integrated Genetics Inc., One Mountain Road, Framingham, MA 01701.* The proto-oncogene product, pp60$^{c\text{-}src}$, is a cellular tyrosine kinase capable of associating with the middle-T antigen of polyomavirus. Examination by site-directed mutagenesis has demonstrated that the kinase and transforming activities of pp60$^{c\text{-}src}$ are regulated by phosphorylation at Tyr 416 (site of autophosphorylation) and at Tyr 527 (major site of phosphorylation *in vivo*). Complex formation with middle-T results in loss of phosphorylation at Tyr 527 and subsequent activation of its tyrosine kinase activity. To investigate specifically the role of the phosphate moiety on these residues, we have simulated its negative charge by replacing the Tyr residues at 416 and 527 with Glu. Double mutants containing either Gln 416 or Glu 416 and Phe 527 were also constructed.

pp60$^{c\text{-}src}$ from platelet-derived growth factor treated cells or from polyomavirus infected cells contains additional sites of Tyr phosphorylation within the amino terminus of the enzyme. However, it is unclear whether these events are a consequence of the observed activation of pp60$^{c\text{-}src}$ or indeed themselves contribute to the activation of pp60$^{c\text{-}src}$. To identify and investigate the roles of these residues in regulating the activities of pp60$^{c\text{-}src}$, site-directed mutagenesis was employed to alter Tyr 90, 92, 131, 136 and 149 to Phe. These amino acids reside within the 18 kD *S. aureus* V8 fragment shown to contain the phosphorylated residues. The catalytic activities of these mutants, their transforming activities and their ability to affect the state of phosphorylation at Tyr 416 and 527 have been examined. These studies should enable an evaluation of the contribution of these events towards cellular transformation by activated pp60$^{c\text{-}src}$ and by middle-T antigen.

E 204 AUTOPHOSPHORYLATION DEPENDENT EXPOSURE OF A REGION IN THE CATALYTIC DOMAIN OF THE EGF RECEPTOR, Gail M. Clinton, Larry A. Compton and Louise E. Kimball, Department of Biochemistry, OHSU, Portland, OR 97201

Protein kinases contain regions of homology, particularly in the kinase domains which function in ATP binding and catalysis. A subdomain we have targeted for investigation is highly conserved in the tyrosine kinase family but only partially conserved in the serine/threonine family of protein kinases suggesting a role in recognition of the different hydroxyamino acid substrates. We prepared antibodies to the sequence His-Arg-Asp-Leu-Ala-Ala-Arg-Asn corresponding to amino acids 811 to 818 of the human EGF receptor. The antibody, purified by peptide affinity chromatography, had no detectable effect on the EGF receptor kinase activity toward exogenous substrates. In striking contrast, when the receptor was autophosphorylated, its kinase activity was inhibited about 60% with 1.5nM and 90% with 3nM antibody. The inhibition was not dependent on ligand binding, was blocked by preincubation of the antibody with peptide, and was not observed using purified IgG from preimmune sera. Consistent with these findings, the kinase domain antibody immunoprecipitated only the autophosphorylated EGF receptor. The specificity of the antibody for the autophosphorylated EGF receptor was eliminated when the protein was denatured. This is the first direct demonstration of a conformational change in the catalytic domain of a tyrosine kinase following autophosphorylation.

E 205 ANTI-CD3 MODULATES PHOSPHORYLATION OF p56 *lck* IN JURKAT T CELLS.

Silvia Danielian, Remi Fagard, Andrés Alcover, Oreste Acuto and Siegmund Fischer, INSERM U.15, 24 rue du fbg. Saint Jacques 75014 Paris.

p56 *lck*, closely related to p60 *src*, is a protein tyrosine kinase that can be detected in normal T lymphocytes and in human lymphomas (Ke37, Jurkat, Molt 4). The abundance of *lck* transcripts in T lymphocytes, suggests that p56 *lck* normally participates in growth regulatory or differentiative pathways unique of this cells. Because Jurkat cells bear a mature T-cell phenotype and can be induced to produce the early signal transduction events in response to CD3-Ti or CD2 stimulation, they are a suitable model system to study the possible implication of p56 *lck* in the signalling pathway of T cells. Stimulation of Jurkat T cells by anti-CD3, anti-clonotypic or anti-CD2 mAbs at concentrations optimal for calcium elevation and PKC stimulation, results in the hyperphosphorylation of p56 *lck* and the appearance of a higher molecular weight form (65 kDa). The diminished mobility of the *lck* gene product is associated with the appearance of additional phosphorylation sites containing phosphoserine. The observed modifications occur within 3' of exposure of these agents. These results raise the issue of the implication of p56 *lck* in the signalling pathway of T-cells.

E 206 DISTINCTIVE EFFECTS OF PHORBOL ESTERS ON ONCOGENIC AND PROTO-ONCOGENIC NEU GENE PRODUCTS, Kunio Dobashi, David B. Weiner and Mark I. Greene. Department of Pathology & Laboratory Medicine, University of Pennsylvania Philadelphia, PA 19104

The products of the neu proto-oncogene, p185 , and the epidermal growth factor receptor (EGFR) are similar and are members of a family of growth factor receptors with tyrosine kinase activity. In the rat, a point mutation producing a single val to glu substitution in the transmembrane domain of p185 converts the neu proto-oncogene to a transforming oncogene. Protein kinase C, which is activated by the tumor promoting phorbol esters has distinct effects on signal transduction of tyrosine kinase receptor proteins. Treatment of cells expressing the EGFR with TPA decreases EGF-stimulated tyrosine kinase activity and DNA synthesis. It has been proposed that this action of TPA results from the phosphorylation of the EGFR by protein kinase C at a critical threonine residue.

Using mouse NIH-3T3 fibroblasts transfected with either the proto-oncogenic or oncogenic rat neu genes, we observed that TPA stimulated the phosphorylation of ser and thr and decreased the FCS-stimulated tyr phosphorylation of proto-oncogenic p185. TPA inhibited the proliferation of cells expressing proto-oncogenic p185. The phosphorylation of oncogenic p185 and the proliferation of cells expressing the oncogenic p185 were not affected by TPA treatment.

These findings suggest that the functional activity of p185 may be modulated by protein kinase C mediated phosphorylation. Most importantly the transmembrane point mutation presented in the oncogenic neu gene may lead to escape from this putative regulatory mechanism.

E 207 MONOCLONAL ANTI-EGF RECEPTOR ANTIBODIES INHIBIT THE GROWTH OF MALIGNANT AND NONMALIGNANT HUMAN MAMMARY EPITHELIAL CELLS, Bruce W. Ennis, Eva M. Valverius, Marc E. Lippman, Francoise Bellot, Richard Kris, Joseph Schlessinger, Hideo Masui, John Mendelsohn, Robert B. Dickson, V.T. Lombardi Cancer Research Center, Georgetown University Medical Center, 3800 Reservoir Rd. NW, Washington, DC 20007 Monoclonal antibodies against the EGF receptor (aEGFR) were tested for their ability to inhibit the growth of 184A1N4 and 184A1N4-T human mammary epithelial cells and MDA-468 human breast cancer cells. Monoclonal aEGFR proved effective at inhibiting cell growth. Four antibodies of 2 different classes were tested: 225 IgG, 108 IgG, 96 IgM and 42 IgM. These antibodies exhibited differential growth inhibitory effectiveness and this correlated with each antibodies ability to compete with EGF for binding to the EGF receptor. Monoclonal 225 IgG and 96 IgM were most effective at inhibiting growth and in competing with EGF for receptor binding. The 184A1N4 and 184A1N4-T cell lines depend on EGF (or TGFα) for anchorage dependent and anchorage independent growth, respectively. Monoclonal aEGFR (2-80nM) inhibited this EGF stimulated growth. Growth inhibition caused by 20nM aEGFR could be incompletely reversed by increasing concentrations (up to 10nM) of EGF. MDA-468 cells overexpress the EGF receptor and are growth stimulated by very low concentrations of EGF or TGFα but they are growth inhibited by high concentrations of these growth factors. MDA-468 cell growth, in the absence of EGF, was inhibited by aEGFR in both anchorage dependent and independent conditions. In the presence of increasing concentrations of EGF (up to 10nM) and 20nM aEGFR MDA-468 cell growth was first stimulated then inhibited as the EGF concentration increased. The results indicate the potential effectiveness of aEGFR in inhibiting the growth of malignant cells that are dependent upon EGF or TGFα for growth.

E 209 CORRELATION OF POOR PROGNOSIS IN BREAST CANCER WITH GROWTH IN NUDE MICE AND AMPLIFICATION/OVEREXPRESSION OF THE HER-2/neu ONCOGENE, Beppino C. Giovanella, Axel Ullrich, Dennis J. Slamon and John S. Stehlin. St. Joseph Hospital Cancer Research Laboratory, Houston, TX 77002, Department of Molecular Biology, Genentech, Inc., South San Francisco, CA 94080, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024. Two hundred and sixty-two primary human breast cancers diagnosed histologically as infiltrating duct cell carcinomas were heterotransplanted subcutaneously in nude Swiss mice. Sixteen of them took and could be serially transplanted. This represents 6.1% of the total. Of the 16 patients with positive takes, 14 were followed for more than 3 years. Nine of them, or 64%, died of the disease within 3 years. The two observed for less than three years are alive with lung and liver metastases, respectively. Of the 246 patients whose tumor did not take in the nudes, 49 or 21% died of the disease within 3 years of tumor removal. Five of ten breast carcinomas growing in the nudes exhibited amplification of the HER-2/neu oncogene. Amplification of this gene has been shown to be associated with poor prognosis in human breast carcinomas.

E 210 IMMUNOCYTOCHEMICAL LOCALIZATION OF EPIDERMAL GROWTH FACTOR RECEPTOR IN MOUSE EMBRYONIC FIBROBLASTS, Dorothy Glaves and Charles E. Wenner*, Department of Experimental Pathology and *Experimental Biology, Roswell Park Memorial Institute, Buffalo, NY 14263. G.Carpenter and S.Cohen (Oncogenes and Growth Factor ed. A.Bradshaw and S.Prentis, (1987)) have considered that ligand-dependent internalization of the EGF-receptor complex may represent a mechanism to facilitate the translocation to the nucleus where it might act directly with the genome. The implication is that the receptor represents the "second messenger" for growth factor-induced mitogenesis. Previous reports of Murthy et.al. (J.C.B. 103:333-338 (1986)) indicated that following EGF introduction, the receptor kinase domain migrates to the perinuclear region. In our studies, immunofluorescent visualization with EGF induced C3H mouse embryonic fibroblasts using EGF receptor antibodies indicate that an EGF challenge results in not only perinuclear labelling but descrete nuclear labelling as well. In addition, there is extensive labelling of fibrous elements which appear to co-localize with cytoskeletal elements suggestive that the EGF receptor follows tracks of filamentous organization (e.g. vimentin, actin or microtubules). If the nuclear localization of the receptor can be confirmed by electron microscopy visualization, the idea that the continual internalization of EGF receptor complexes is obligatory for DNA synthesis becomes more tenable, particularly in view of the reported requirement by Schecter et.al. (PNAS 75:5788-5791 (1978)) of persistent occupation of high affinity cell surface receptors for biological activity.

E 211 MODULATION OF c-erbA PROTO-ONCOGENE EXPRESSION BY GLUCOCORTICOIDS IN S49 LYMPHOMA CELLS. Alberto Gulino, Marella Maroder, Alessandra Vacca, Elisa Petrangeli and Luigi Frati,Department of Experimental Medicine, Univ. La Sapienza, Rome and Department of Sciences, Biotechnology and Biometrics, Univ. of L'Aquila, Italy.
Glucocorticoid hormones are known to inhibit the growth of lymphoma cells. Since several proto-oncogenes have been shown to play a role in the regulation of cell proliferation, we studied the effect of glucocorticoids on c-myc and c-erbA proto-oncogene expression. Treatment of S49 lymphoma cells with 1 uM dexamethasone decreased (3H)Thymidine uptake between 6 and 24 h after hormone treatment (80-90% decrease 24h after treatment). Northern blot analysis of RNA extracted from dexamethasone-treated cells showed a significant decrease of c-myc mRNA levels between 3 h and 24 h after hormone treatment. A 6.0 kb and 2.7 kb mRNA bands hybridizing with a human c-erbA cDNA probe (Weinberger et al. Nature 324:641, 1986) were observed in S49 cells. Cycloheximide treatment of S49 cells slightly increased the levels of c-erbA mRNA. The 2.7 kb c-erbA mRNA levels were increased between 8 h and 24 h after dexamethasone treatment. This dexamethasone-induced increase of c-erbA mRNA was partially abolished by cycloheximide treatment, suggesting that ongoing protein synthesis is necessary to elicit this hormone effect. The levels of c-myc and of c-erbA mRNAs were not modified by dexamethasone in a clone of the MCF-7 epithelial breast cancer cell line and in MLA 144 lymphoma cell line whose proliferation rates were not affected by the drug. We conclude that the decrease of cell proliferation induced by dexamethasone is associated with a decrease of c-myc mRNA levels and an increase of c-erbA mRNA in S49 lymphoma cells, suggesting that such oncogenes could be involved in the control of cell proliferation.

E 212 CHARACTERIZATION OF THE DELETION OF THE RB GENE IN RETINOBLASTOMA AND OSTEOSARCOMA, Tomoko Hashimoto, Rei Takahashi, *Yuen-Kai T. Fung, Hong-Ji Xu, Sandy Gunnell, and William F. Benedict, Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381, * Childrens Hospital of Los Angeles, Los Angeles, CA 90027
The retinoblastoma (Rb) gene is a putative cancer suppressor gene which is involved in various human cancers. We have isolated the genomic locus of the Rb gene and have been able to clone twelve single-copy fragments which were derived from intron regions of the gene. In order to specify the deletion sites and polymorphic regions of the Rb locus, we used these probes as well as cDNA probes for detailed characterization of the structural changes of the Rb locus in retinoblastomas and osteosarcomas. We also analyzed the specific internal deletions of two retinoblastomas and one osteosarcoma by constructing genomic libraries from these mutant cells and by the PCR technique. The significance of the structural deletions will be discussed as it relates to abnormal transcript and gene production.

E 213 THE USE OF EGF RECEPTORS AND AN ALVEOLAR DIFFERENTIATION MARKER TO PREDICT A POOR SURVIVAL IN LUNG CANCER, F. Hendler, A. Shum-Siu, L. Nanu, D. Yuan, B. Ozanne, University of Louisville, Louisville, KY 40292 & University of Texas Health Science Center, Dallas TX 75235.

We have studied EGF receptor in 44 biopsy specimens from primary lung cancers using a quantitative radioimmunoassay on tissue sections. All squamous tumors studied have increased EGF receptors with a range of from 1.5 to 20 times the level detected in normal skin biopsy specimens. This subset of tumors are more undifferentiated tumors when compared to those with 1.5 to 3 fold increased receptor ($p < .04$). Overexpression of the EGF receptor is associated with a poor survival ($p \leq .02$). When the EGF receptor is greater than 2.5 fold normal skin, the median survival is 9 months; if less than 3 fold, median survival is 25 months. Those patients with EGF receptor values less than 3 fold normal skin can be subdivided into groups: those that do as poorly with a similar survival time to that of patients with very high EGF receptor levels and those who do well with a median survival of 57+ months. Specimens were similarly evaluated for the presence of an alveolar differentiation antigen, H71. Antigen positive specimens have an excellent prognosis whereas antigen negative have a prognosis similar to that in association with overexpression of the EGF receptor ($p \leq .002$). These markers are syngeristic in that patients lacking in H71 with increased EGF receptor have an even greater liklihood of doing poorly ($p \leq .0002$). Studies are underway to determine other biologic events which are affecting survival in patients with lower levels of EGF receptor.

E 214 THE RETINOBLASTOMA SUSCEPTIBILITY GENE IS FREQUENTLY REARRANGED AND RARELY EXPRESSED IN SMALL CELL LUNG CANCER. Charles H. Hensel[1], Chih-Lin Hsieh[1], Adi F. Gazdar[2], Bruce E. Johnson[2], Alan Y. Sakaguchi[1], Susan L. Naylor[1], Wen-Hwa Lee[3], and Eva Y.-H. Pan-Lee[3], [1]The University of Texas Health Science Center at San Antonio, [2]NCI Navy Oncology Branch, [3]University of California, San Diego.

Previous results from our laboratory have shown frequent loss of alleles from chromosomes 3, 13 and 17 in small cell lung cancer (SCLC). Since the retinoblastoma susceptibility gene locus RB-1 is encoded on chromosome 13, band q14, we examined SCLC tumor and matched normal tissue DNA for loss of DNA from the RB-1 locus. Six of six patients whose DNA was heterozygous for an RFLP detected by the RB-1 probe p6NRO.5 showed loss of one allele in SCLC tumor DNA. In addition, one tumor and one SCLC cell line (H865) showed homozygous deletions within the RB-1 gene. Markers more distal to the RB-1 locus, at 13q22-31 (D13S4) and 13q33-qter (D13S3), showed loss in 8 of 11 and 6 of 15 cases respectively. Several SCLC tumor cell lines also were examined for expression of the 4.7kb RB-1 mRNA. Lines H209 and H592 expressed moderate levels of RB-1 mRNA; line H1284 expressed a lower level of RB-1 mRNA; all other lines expressed only trace or undetectable amount of RB-1 mRNA. In vivo labelling of 14 SCLC cell lines showed that only H209 and H592 produced detectable quantities of $pp110^{RB}$, the protein encoded within the RB-1 gene. However, the protein seen in H209 was not phosphorylated and the protein in H592 was not phosphorylated as highly as $pp110^{RB}$ from control cells. Surprisingly, H1284, which expressed a moderate level of RB-1 mRNA, did not express detectable $pp110^{RB}$. These data indicate that the RB-1 gene may play a role in the etiology of SCLC.

E 215 EGF RECEPTORS EXPRESSED IN K562 HAVE CONSTITUTIVELY ENHANCED TYROSINE KINASE ACTIVITY. J. Justin Hsuan, Hamish Allen*, S. Clark#, R. Maziarz†, MD Waterfield, RA Flavell§ and John Haley. Ludwig Inst. for Cancer Research, Middlesex Hosp./UCL, London, UK; *National Inst. for Medical Research, London, UK; #Walter and Eliza Hall Inst. of Medical Research, Melbourne, Australia; †Dana-Farber Cancer Inst., Boston, MA02115, USA; §Yale Univ. School of Medicine, New Haven CT 06510, USA.

The EGF receptor is a 170kD transmembrane glycoprotein that transduces a ligand-dependent mitogenic signal in a variety of nonhaematopoietic cell types. The receptor tyrosine kinase activity is critical to this signalling though both the mechanism of kinase activation and the immediate intracellular pathways that are directly regulated by tyrosine phosphorylation remain obscure.

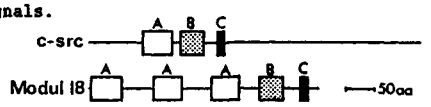
In order to investigate of the role of MHC class I association with EGF receptors we expressed the human EGF receptor in the K562 human erythroleukemic cell line. No evidence for an association that modulates ligand affinity or receptor internalization was found, but the receptor showed enhanced specific tyrosine kinase activity both *in vitro* and *in vivo*. This activity was not caused by either ligand binding or receptor truncation. Metabolic labeling studies have revealed a unique phosphothreonine peptide and results will be presented from an investigation of the role of this modification in the regulation of EGF receptor tyrosine kinase activity in these cells.

E 216 THE trk GENE FAMILY OF GROWTH FACTOR RECEPTORS, Rudiger Klein*§, Diego Pulido*, Francois Coulier*, Toshiaki Koda*§, Dionisio Martin-Zanca* and Mariano Barbacid*§. Basic Research Program, Frederick Cancer Research Facility, Frederick, MD 21701*, and Dept. of Molecular Biology, The Squibb Institute for Medical Research, Princeton, NJ 08543§.

The trk locus was identified as the result of oncogenic activation of its tyrosine kinase domain by a genomic rearrangement that fused it to tropomyosin sequences in a human colon carcinoma (Nature 319:743, 1986). Molecular characterization of a cDNA clone of the human trk proto-oncogene indicated that this locus codes for a novel tyrosine kinase cell surface receptor for an, as yet, unidentified ligand (MCB, in press). We now report that the trk locus is a member of an evolutionarily conserved family of growth factor receptors. We have isolated a highly related gene from a mouse brain cDNA library. This gene, designated trkB, shares 88% amino acid homology with the tyrosine kinase domain of the human trk gp140 protein. This homology, however, is only 50% in the transmembrane and extracellular domains indicating that the trkB gene product is likely to recognize a different ligand. The trk and trkB genes may have evolved from a single ancestor locus, since the Drosophila genome contains a trk-related gene, Dtrk, which shares 62% amino acid homology with the tyrosine kinase domains of either human trk and mouse trkB. Preliminary studies in both mice and Drosophila indicate that this family of growth factor receptors is preferentially expressed during embryonic development. In addition, low levels of expression of the trk locus have been found in adult brain tissues. A similar, although less restricted pattern of expression, has been observed for the mouse trkB gene. These results indicate that trk is a family of growth factor receptors potentially implicated in embryonic development.

E 217 Modul18 A NOVEL PROTEIN WITH SEQUENCE SIMILARITY TO scr RELATED TYROSINE KINASES, Jurgen M. Lehmann, Christine Sers, Gert Riethmuller and Judith P. Johnson, Inst. for Immunology, University of Munich, D-8000 Munich 2, F.R. of Germany

cDNA clones encoding Modul18, a protein of 377 amino acids with a calculated M_r of 42,750 daltons was isolated from a human melanoma cDNA expression library. These clones identified a single 2.0kb mRNA band which was found in a wide variety of cell lines as tested by Northern blot analyses. Southern blot analyses suggested the presence of at least two closely related genes. Hydrophobicity analysis of the predicted amino acid sequence showed no indication of a signal peptide or a membrane spanning region. The amino acid sequence shows a high sequence similarity to the non-catalytic domain of non-receptor tyrosine kinases. However in contrast to the tyrosine kinases the catalytic domain is totally absent from the Modul18 protein. In addition a region in the non-catalytic domain refered as region A is present 3 times. The absence of the catalytic tyrosine kinase domain suggests that Modul18 is involved in direct or indirect modulation of endogenous tyrosine kinases and therefore involved in the transduction or control of mitogenic and developmental signals.



Linear representation of regions displaying sequence similarity in c-src and Modul18

E 218 A CHIMERIC EGF-R/*neu* PROTO-ONCOGENE ALLOWS EGF TO REGULATE *neu* TYROSINE KINASE AND CELL TRANSFORMATION. Heikki Lehväslaiho, Laura Lehtola, Lea Sistonen and Kari Alitalo, Department of Virology, University of Helsinki, Haartmaninkatu 3, SF-00290 Helsinki, FINLAND

The *neu* oncogene, characterized by Weinberg and colleagues is a transforming gene found in ethylnitrosourea-induced rat neuro/glioblastomas; its human homologue has been termed *erb*B2 or HER2 because of its close homology with the epidermal growth factor receptor (EGF-R) gene (c-*erb*B1). Expression of the rat *neu* oncogene is sufficient for transformation of mouse NIH/3T3 fibroblasts in culture and for the development of mammary carcinomas in transgenic mice, but the *neu* proto-oncogene has not been associated with cell transformation. – We constructed a vector for expression of a chimeric cDNA and hybrid protein consisting of the EGF-R extracellular, transmembrane and protein kinase C-substrate domains linked to the intracellular tyrosine kinase and carboxyl terminal domain of the rat *neu* gene. Upon transfection, NIH/3T3 cells gave rise to EGF-R antigen-positive cell clones with various amounts of specific EGF binding. The clones also expressed of EGF-R and *neu* antigens at the cell surface and a chimeric protein of the expected apparent molecular weight could be precipitated with both *neu*- and EGF-receptor antibodies from these cells. Within one minute of its addition, EGF caused tyrosine phosphorylation of the chimeric receptor protein but not of an amplified *neu* protein in NIH/3T3 cells and led to a transformed cell morphology and growth in soft agar. These results suggest that the *neu* proto-oncogene can transform cells only in the presence of a ligand which stimulates its tyrosine kinase activity and provide the first model for studies of the function of the *neu* tyrosine kinase.

E 219 MONOCLONAL ANTIBODY RECOGNIZING THE HER2 GENE PRODUCT HAS ANTIPROLIFERATIVE EFFECTS IN VITRO AND SENSITIZES HUMAN BREAST TUMOR CELLS TO TUMOR NECROSIS FACTOR, Gail D. Lewis, Robert M. Hudziak, Marcy Winget, Brian Fendly, Axel Ullrich and Michael Sheppard, Genentech, Inc., South San Francisco, CA 94080

The HER2 gene encodes a cell surface glycoprotein, $p185^{HER2}$, with homology to the epidermal growth factor receptor. Amplification of this gene occurs in approximately 30% of human primary breast tumors and is correlated with a poor prognosis in node-positive patients. High levels of expression of $p185^{HER2}$ will transform mouse NIH 3T3 cells. These cells simultaneously become resistant to cytotoxicity mediated by TNF-α and activated macrophages. Monoclonal antibodies directed against the extracellular domain of the HER2 gene product were prepared. Seven of nine antibodies tested will inhibit the growth of the human breast tumor cell line, SK-BR-3. One monoclonal antibody, designated 4D5, was further investigated. Antibody 4D5 recognizes the extracellular domain of $p185^{HER2}$ and does not cross-react with the EGF receptor. It will inhibit the growth of breast tumor cell lines overexpressing $p185^{HER2}$, but not nontransformed mammary gland epithelial cells or the bladder carcinoma cell line T24. Treatment of two breast tumor cell lines overexpressing $p185^{HER2}$ with antibody 4D5 increases their sensitivity to the cytotoxic effects of TNFα. These results suggest a role for HER2 gene expression not only in tumor cell growth, but also in tumor progression by allowing malignant cells to evade the antitumor surveillance of activated macrophages.

E 220 CHARACTERIZATION OF A RECOMBINANT ABELSON TYROSINE PROTEIN KINASE (TPK), Nicholas B. Lydon, Ben Adams, Jens F. Poschet, Alois Gutzwiller and Alex Matter, Oncology and Virology, Pharmaceutical Research Department, CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland. Bacterial expression plasmids containing sequences derived from Abelson murine leukemia virus (A-MuLV) were constructed such that the gag region of the v-abl gene was replaced by a sequence encoding the IgG-binding domain of the S. aureus protein A gene. All vectors which included the 5′ 1.2 kb of v-abl sequence encode fusion proteins with TPK activity when expressed in E.coli. pabl HP, a fusion protein encoding the previously defined minimal transforming region of v-abl was purified and characterized. The Km_5 for ATP was 21.2 μM at saturating concentrations of [Val^5]-angiotensin II. The Km for [Val^5]-angiotensin II at saturating concentrations of ATP was 3.8 μM. The turnover number for the pabl HP kinase, measured at 20°C, was 62 μmol min^{-1} μmol^{-1}. The substrate specificity of the pabl HP kinase was studied using synthetic peptide analogs of the sequence around the phosphorylation site of $pp60^{src}$ and analogs of angiotensin II. The specificity constant for the phosphorylation of the 13 residue peptide Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Tyr-Ala-Ala-Arg-Gly was 4.5 x higher than that observed with [Val^5]-angiotensin II. This E.coli expression system provides an experimental method for enzymatic and structural studies to be carried out on the TPK domain of v-abl.

E 221 MOLECULAR BIOLOGY OF GAP AND ITS INTERACTION WITH ras p21. M.S. Marshall, W.S. Hill, U.S. Vogel, R.A.F. Dixon, M.D. Schaber, R.E. Diehl, A. Ng, E.M. Scolnick, I.S. Sigal and J.B. Gibbs, Department of Molecular Biology, Merck Sharp and Dohme Research Laboratories, West Point, PA 19486. Recent work by Trahey and McCormick (Science,238, 542, 1987) has identified a novel protein called GAP (GTPase Activating Protein) which stimulates the GTPase activity of normal ras p21, but not that of the oncogenic variants. We have recently reportedthe purification of a 125 kDa protein from bovine brain with GAP activity. (Gibbs et al. PNAS 85,5026,1988). Using a kinetic competition assay to study the physical interaction between GAP and ras p21, we have shown that both normal and oncogenic ras p21 interact with GAP when complexed to GTP. The complete amino acid sequence of GAP has been determined by sequencing the corresponding cDNA (Vogel et al. Nature 335,90,1988). Regions of amino acid similarity exist between GAP and regions conserved between phospholipase C-145, the crk oncogene product, and the nonreceptor tyrosine kinases. Deletion analysis has identified a domain of GAP sufficient for GAP-stimulated p21 GTPase activity. Current efforts involving expression of GAP in a variety of systems are aimed at elucidating whether GAP is an upstream regulatory protein or the downstream target of ras p21.

E 222 PHASE I CLINICAL TRIAL WITH ANTI-EGF RECEPTOR MONOCLONAL ANTIBODY (mAb), J. Mendelsohn, C. Divgi, S. Yeh, H. Masui, R. Gralla, M. Kris, F. Real, M. Unger, S. Schweighardt, R. Bartholomew, G. David, A. Goldenberg, and S. Welt., Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY 10021 and Hybritech, Inc, La Jolla, CA 92037. mAb 225 IgG1 binds to the human EGF receptor with an affinity comparable to EGF, competes with EGF for binding to the receptor, prevents EGF-induced activation of receptor tyrosine kinase, and inhibits the proliferation of a number of human tumor cells bearing high numbers of EGF receptors in culture and in nude mouse xenografts. A431 xenografts bearing high numbers of EGF receptors can be imaged with ^{111}In-labeled mAb. We postulate that mAb-induced inhibition of proliferation is due to interference with an autocrine loop involving TGF alpha. Increased expression of EGF receptors and production of TGF alpha has been documented in many human tumors and tumor cell lines. Phase I trials were initiated in patients with advanced squamous cell carcinoma of the lung, which consistently expresses high numbers of EGF receptors. ^{111}In-labeled 225 IgG1 was given by single intravenous infusion and doses were escalated in groups of three patients. Preliminary results show no toxicity from doses up to 40 mg. Tumor visualization was seen to be dose-related, and significant liver and bowel isotope uptake was also observed. Further dose escalation is proceeding, and pharmacokinetic data will be obtained. These preliminary observations suggest that 225 IgG1 may be useful for imaging tumors which bear increased numbers of EGF receptors, and they raise the possibility that therapy with antireceptor mAbs may be a worthwhile area for investigation.

E 223 ALTERATIONS IN EGF RECEPTOR TYROSINE KINASE SUBSTRATE SPECIFICITY BY TUMOR PROMOTERS, Fred H. Mermelstein, Tanveer F. Abidi and Jeffrey D. Laskin, Department of Environmental and Community Medicine, UMDNJ-Robert W. Johnson Medical School, Piscataway, NJ 08854
The ability of chemicals to alter the response of epithelial cells to growth factors is an important mechanism of tumor promotion and carcinogenesis. We found that the tumor promoters 12-O-tetradecanoylphorbol 13-acetate (TPA) and photoactivated psoralens (PUVA) are potent inhibitors of EGF receptor binding and EGF stimulated EGF receptor tyrosine kinase activity. Using the human epidermal cell line A431, both of these promoters were found to stimulate phosphorylation of the EGF receptor. While TPA enhanced both serine and threonine phosphorylation, PUVA selectively stimulates serine phosphorylation of the receptor. This resulted in a change in the ability of the EGF receptor to autophosphorylate. Maximal inhibition of intrinsic EGF receptor tyrosine kinase activity by TPA and PUVA was 90% and 50%, respectively. Modification of the receptor by TPA and PUVA also resulted in a change in substrate specificity of the EGF receptor. We also found that PUVA, but not TPA, selectively stimulated phosphorylation of a 32 kD membrane bound protein. Taken together our data suggests that TPA and PUVA modulate EGF receptor function by distinct mechanisms. Supported by NIH grant ES 03647.

E 224 USE OF GAG-erbB FUSION PROTEINS TO STUDY THE KINASE ACTIVITY OF erbB, N. Nair[1], R.J. Davis[2], H.L. Robinson[1]. Departments of Pathology[1] and Biochemistry[2], University of Massachusetts Medical Center, Worcester, MA, 01655. To study the potential role of the kinase activity of the erbB region of the EGF receptor in transformation, a defective avian retrovirus expressing a gag-erbB fusion protein has been constructed. The gag-erbB protein contains 417 amino acids of gag fused to 535 amino acids encompassing ~60 amino acids external to the transmembrane domain, the kinase domain and a truncated autophosphorylation domain of the chicken EGF receptor. The truncation in the autophosphorylation domain is 38 amino acids after tyrosine 1068, the homolog of the P3 autophosphorylation site of the human EGF receptor. The defective virus did not transform cultured fibroblasts but was associated with the induction of erythroblastosis in K28 chickens. The fusion protein autophosphorylates tyrosine 1068 following immunoprecipitation with a polyclonal anti-gag antiserum. The fusion protein phosphorylates src peptide with the Km for the peptide being ~ 1.9mM. Tests to maximize ATP concentration have revealed marked increases in activity with increasing levels of ATP (from 1 um to 50 um) that are inconsistent with expected Michaelis-Menten kinetics. These results suggest that the kinase activity of the gag-erbB fusion protein maybe strongly activated by autophosphorylation, a possibility which is under test.

E 225 THE ACTIVATION OF IL6 INDUCIBLE GENES THROUGH A NOVEL SIGNAL TRANSDUCTION PATHWAY. Koichi Nakajima and Randolph Wall, UCLA, Department of Microbiology and Immunology, The Molecular Biology Institute,Los Angeles, CA 90024.
Interleukin 6 (IL6) is multifunctional factor acting on different lineages of cells. IL6 functions as a growth factor for myeloma/hybridoma cells and induces the differentiation of activated B cells and pheochromocytoma Pc12 cells. We have analyzed the signal transduction pathways activated by IL6 in a IL6-dependent hybridoma, MH60. BSF2, by examining the pattern of mRNA expressed from the activation responsive genes (Tis11, B37, c-*myc*, ODC, Egr-1) in response to IL6. The role of protein kinases in the signaling pathway was investigated using the inhibitors H7 or HerbimycinA, which have inhibitory effects on the growth of MH60 cells and are known to inhibit protein kinase C (PKC) and tyrosine kinases respectively. IL6 induced expression of Tis11, B37, ODC and c-*myc* but not of Egr-1. The activation of these genes with IL6 was inhibited by H7, not by HerbimycinA. The H7-sensitive pathway(s) can be distinguished from PKC or cAMP dependent protein kinase pathways. The PKC activator, TPA, and /or cAMP analogue, dibutyryl cAMP, did not change the IL6 dependency of MH60 cells. A selective PKC inhibitor, sphingosine, does not inhibit Tis11 gene expression. IL6 also induces Tis11 gene expression under conditions where PKC has been depleted by TPA pretreatment. Egr-1 was activated by TPA, while dibutyryl cAMP had no effect on IL6 induction of Tis11, B37 and c-*myc*. These results suggest that IL6 activates a set of genes important for cell growth through an H7-sensitive pathway which is distinct from activation pathways utilizing PKC, cAMP dependent kinases or tyrosine kinase.

E 226 **A SWISS 3T3 VARIANT RESISTANT TO THE MITOGENIC EFFECTS OF TUMOR PROMOTERS CANNOT BE TRANSFORMED BY SRC.** *Mukund Nori, Laura K. Shawver and Michael J. Weber, Dept. of Microbiology, Univ. of Virginia School of Medicine, Charlottesville, VA 22908.*
We are developing genetic tools to analyze intracellular signalling by $pp60^{v\text{-}src}$, and to this end have examined the ability of the *src* oncogene to transform cells defective in aspects of normal mitogenesis. Here we report that TNR9-3T3 cells, a variant of Swiss 3T3 cells which is mitogenically non-responsive to tumor promoters such as TPA, cannot be transformed by *src*. TNR9 cells and parental 3T3 cells were infected with a murine recombinant retrovirus carrying the v-*src* and *neo*^{r} genes and selected for resistance to G418. The virus was able to infect both cell types, but could transform only the 3T3 cells, and not the TNR9 cells, as judged by cell morphology, hexose uptake and growth in soft agar. Most infected clones of TNR9 cells showed only low levels of expression of active $pp60^{v\text{-}src}$, as determined by Western blot analysis using antibodies against $pp60^{v\text{-}src}$ or against phosphotyrosine. However, a few clones displayed tyrosine phosphorylation levels comparable to those obtained in some transformed 3T3 clones. Preliminary results point to the possibilty of specific phosphorylation defects in these clones. Co-transfection experiments of v-*src* with *neo*^{r} suggested that expression of v-*src* was selected against in the TNR9 background. Expression of v-*myc*, while not transforming for these cells, could compensate for this effect, and permitted the TNR9 cells to become transformed by v-*src*. The TNR9 cells were found not to be resistant to all oncogenes: they were readily transformed by murine retroviruses carrying the v-*abl* or v-*ras* oncogenes. Our results suggest that transformation by *src* and mitogenesis by TPA share a common step in intracellular signalling.

E 227 EARLY ACTIVATION OF THE ENDOGENOUS $pp60^{src}$ KINASE ACTIVITY DURING NEURONAL DIFFERENTIATION OF CULTURED HUMAN NEUROBLASTOMA CELLS. S Påhlman, C Bjelfman, G Meyerson, K Mellström, U Hammerling, Department of Pathology, University of Uppsala, S-751 85 Uppsala, Sweden. $pp60^{src}$ is highly expressed in neurons and neuroendocrine cells. While neuronally differentiated cells express a src product ($pp60^{n\text{-}src}$) which is structurally distinct from the normal cellular src protein ($pp60^{c\text{-}src}$), neuroendocrine cells appear to express only $pp60^{c\text{-}src}$. Since $pp60^{src}$ has been implicated in the control of cell differentiation, $pp60^{n\text{-}src}$ expression might be fundamental in the regulation of neuronal differentiation. The human SH-SY5Y neuroblastoma cells express both forms of $pp60^{src}$ (approx. 50% of each). As these cells can be induced to differentiate further along a neuronal pathway, we are using this model to investigate a potential role of $pp60^{n\text{-}src/c\text{-}src}$ in neuronal differentiation. Phenotypical changes of phorbolester-induced functionally differentiated SH-SY5Y cells include growth inhibition, formation of neurites and growth cone-like structures. The membrane potential increases and these cells can be depolarized by acetylcholine which triggers the release of the stored noradrenalin. There was no change in the $pp60^{n\text{-}src/c\text{-}src}$ ratio upon differentiation. However, with orthovanadate present during lysis, an increase in specific src-kinase activity was detected 4-6 h after induction. The activation appeared during the same time interval as the cells formed neurites with growth cones and a growth cone associated protein, GAP 43, was induced. After 4 days of treatment the cells had 3-4 times higher src kinase activity than control cells, while prolonged treatment reduced this difference. Our results are compatible with a role of $pp60^{src}$ in the control of late neuronal differentiation but do not indicate whether either or both of the two src forms are involved.

E 228 POSSIBLE ROLE FOR INTERSPERSED REPETITIVE SEQUENCES IN NEGATIVE REGULATION OF CELL GROWTH, Kazuichi Sakamoto, Tazuko H. Howard, C. Michael Fordis, Christopher D. Corsico and Bruce H. Howard, Laboratory of Molecular Biology, NIH, NCI, Bethesda, MD 20892
To study mechanisms by which mammalian cell growth can be suppressed, we have performed DNA-mediated gene transfer experiments in which candidate growth inhibitory genes are co-transfected with a cell surface marker plasmid. In our experiments, expression of the surface marker(the Tac subunit of the human IL-2 receptor) is used to purify transiently transfected cells by magnetic affinity cell sorting. DNA replication in the transfected cells is estimated by measuring incorporation of ^{3}H-thymidine per transfected cell. Our results suggest the possibility that some interspersed repetitive sequence (IRS) elements have the capacity to suppress DNA replication of recipient HeLa cells. Several clones of the Alu family and 7SL RNA gene show up to about 50% inhibition of DNA replication as compared to control DNAs. Mutational analysis indicates that the internal RNA polymerase III promoter (Box B) is necessary, if not sufficient, for such activity. Our current hypothesis concerning the possibility that RNAs transcribed from these IRS may negatively regulate cell growth will be discussed.

E 229 INHIBITION OF pp60$^{c\text{-}src}$ KINASE ACTIVITY AND c-*myc* EXPRESSION BY HERBIMYCIN A IN HUMAN MEDULLOBLASTOMA CELLS, Hideyuki Saya, Donald A. Ross, Donald A. Tinker and Victor A. Levin, Department of Neuro-oncology, M. D. Anderson Cancer Center, Houston, TX 77030 and Department of Neurological Surgery, University of California, San Francio, CA 94143. Medulloblastoma, one of the most common pediatric malignancies of the central nervous system, was found to possess c-*src* protein with a high level of phosphotyrosine kinase activity, as well as overexpression of c-*myc* oncogene. We observed that herbimycin A, an inhibitor of pp60^{src} kinase, reduced cell growth and produced neuron-like morphological changes in human medulloblastoma (TE671) cells. It also reduced the *in vitro* autophosphorylation of the immunoprecipitated pp60$^{c\text{-}src}$ although the level of pp60$^{c\text{-}src}$ protein did not change. We assume that the structure of pp60$^{c\text{-}src}$ kinase might be modified and inactivated by herbimycin A *in cellulo* . Interestingly, glioblastoma cells, which have low pp60 $^{c\text{-}src}$ kinase activity, were less sensitive to herbimycin A. Herbimycin A also reduced the level of c-*myc* mRNA in TE671 cells approximately 12 h after administration. Electron microscopic observation showed the remarkable alteration of cytoplasmic structures in herbimycin A treated TE671 cells. In particular, swelling of mitochondrial crestae and structural changes in rough endoplasmic reticulum imply a cellular metabolic disorder. We conclude that pp60$^{c\text{-}src}$ kinase may play a role in the proliferation of medulloblastoma and that further consideration should be given to herbimycin A as a potential treatment for this tumor.

E 230 SIMIAN VIRUS 40-INDUCED TRANSFORMATION MAY BE MEDIATED BY INDUCTION OR ACTIVATION OF A PROTEIN KINASE, Karl H. Scheidtmann and Angelika Haber, Institute for Immunobiology, University of Freiburg, FRG. The cellular protein p53 has been ascribed a key role in simian virus 40 (SV40)-induced transformation. This protein is presumably involved in cell cycle control in normal cells. SV40 infection might lead to its oncogenic activation either by interaction with the viral T-antigen, by overexpression, or other alterations. We have followed changes in the state of phosphorylation of p53 from normal versus SV40-infected or transformed rat cells. In normal cells, p53 was hardly phosphorylated, perhaps only in a subfraction. Upon infection or transformation, a quantitative and qualitative increase in phosphorylation was observed as revealed by two-dimensional phosphopeptide analyses. Enhanced phosphorylation led to conversion to a second form of p53. These phenomena were not seen with transformation-defective mutants. We suggest (i) that SV40 T-antigen induces or activates a protein kinase onesubstrate of which is p53, (ii) that transformation-defective mutants are impaired in kinase induction (activation), and (iii) that either the SV40-induced kinase or a certain phosphorylation state of p53 (and other proteins) might be important for transformation.
Supported by Deutsche Forschungsgemeinschaft through SFB 31.

E 231 DIFFERENTIAL SCREENING REVEALS REDUCED TRANSCRIPTION OF A MITOCHONDRIAL GENE IN TUMOURS ASSOCIATED WITH 11p REARRANGEMENTS: WILM'S TUMOUR AND HEPATOBLASTOMA, M.H. Little and P.J. Smith, Queensland Institute of Medical Research and Department of Pathology, University of Queensland, Herston, 4006, Australia

Tumorigenesis in Wilm's tumour (WT) and hepatoblastoma (HB) is hypothesized to involve loss or inactivation of sequences of 11p. These sequences may act by either (i) down-regulating cellular proliferation factors or (ii) inducing tissue differentiation factors in the normal tissue. In either case, an effective homozygous loss of these sequences in WT or HB should lead to differential expression of unknown genes when the tumour is compared with its normal tissue analogue.

To investigate this, cDNA libraries were constructed from normal kidney adjacent to a WT. Inserts were blunt-end ligated into HincII- cut pGEM1 vector and replica filters were screened using ^{32}p-labelled cDNA synthesized from the kidney and Wt poly A+mRNA. 3 rounds of screening of a library of 12,500 clones identified 9 clones overabundant in the kidney. The largest clone was 760 bp with a poly A+ tail. Hybridization of this clone with mRNA from the original WT/kidney revealed at 710-725bp transcript less abundant in the tumour. Genebank analysis after dideoxysequencing identified this clone as human cytochrome oxidase subunit 2 (CYOX2; 683bp).

3 other WT/kidney pairs also showed reduced CYOX2 mRNA in the tumour, as did an HB from a Beckwith-Wiedemann syndrome patient when compared to autologous liver. We have previously shown 11p allele rearrangements in this HB.

Mitochondrially-encoded CYOX2 functions to bind with nuclearly-encoded cytochrome c and preliminary evidence for down-regulation of a non-nuclear gene in embryonal tumours associated with 11p allele rearrangements.

E 232 **MODULATION OF GROWTH AND EPIDERMAL GROWTH FACTOR RECEPTOR ACTIVITY BY RETINOIC ACID IN GLIOMA CELLS,** **Peter A. Steck, Reuben Lotan, W.K.Alfred Yung, Department of Neuro-oncology and Tumor Biology, M.D.Anderson Cancer Center, Houston, TX 77030.** **The growth inhibitory activity of retinoic acid (RA) was heterogeneous (ID50 10^{-7}-< 10^{-5}M) among eight cultured human glioma cell lines examined. The expression of epidermal growth factor receptor (EGF-R) was not effected by RA treatment, although some sensitive cell lines revealed a slight decrease in receptor affinity. In contrast the tyrosine-kinase activity of EGF-R in the RA sensitive cells was shown to be inhibited in a dose-dependent fashion which appeared to correlate with the RA induced growth inhibition of the cells. The autophosphorylation of RA-treated or untreated EGF-R occurred on similar amino acid residues, albeit at different levels. No alterations in EGF-R activity was seen in RA insensitive cells. The tyrosine kinase activity inhibition appeared to be independent of protein kinase C activity. Also, no alterations were observed between RA-sensitive or insensitive cells in their ability to bind or internize EGF, and degrade EGF-R. However, alterations in associated glycoconjugates of EGF-R was found among the sensitive and insensitive cells. These results suggest RA growth inhibition may be mediated, at least in part, by alterations in EGF-R activity and structure.**

E 233 The structure and expression of ribosomal protein S6 kinases. L.J. Sweet, D. Alcorta, E. Erikson*, S.W. Jones, R.L. Erikson. Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA *Department of Pharmacology, University of Colorado Medical School, Denver, CO.

We have previously described a cDNA corresponding to a mRNA for a Xenopus ribosomal protein S6 kinase (Jones, et al (1988) Proc. Nat'l. Acad. Sci. 85, 3377). The protein predicted by the sequence of this cDNA has two apparent kinase domains that are each similar to distinct protein kinases. When the Xenopus clone was used to screen avian and murine cDNA libraries one avian and two distinct murine homologues were identified. The protein kinase sequences predicted by these clones are all remarkably similar. The mRNAs for these proteins are abundantly expressed in actively dividing tissue suggesting the enzymes may have a role in cell proliferation.

In animal cells S6 kinase activity is stimulated by a wide variety of mitogenic agents such as serum. Antiserum was raised against the protein products of the cDNA clones expressed in bacteria and shown to specifically immunoprecipate serum-stimulated protein kinase activity that can be measured in immune complex assays. In addition, the antiserum immunoprecipates a protein from cell extracts with a similar molecular weight to a polypeptide generated from the cDNA clones by cell-free transcription-translation. We are now using these reagents to investigate the possible mechanism(s) of activation of this highly conserved class of enzymes.

E 234 TRANSCRIPTIONAL ACTIVATION OF *lck* EXPRESSION IN HUMAN TUMOR CELL LINES. Oliver Sartor[1], Francoise Gregory[1], Nancy Smyth Templeton[2], Shashi Pawar[3], Roger Perlmutter[3], and Neal Rosen[2]. NCI, Bethesda, MD[1]; Lombardi Cancer Center, Georgetown University, Washington, DC[2]; HHMI, University of Washington, Seattle, WA[3]. The *lck* proto-oncogene is a member of a family of tyrosine kinases closely related to c-*src*. In normal tissue, it is expressed exclusively in lymphoid cells. *lck* mRNA is expressed in lymphoid tumor cell lines as well, but can also be detected in some colon and small cell lung carcinoma cell lines. This latter ectopic expression is not associated with amplification or rearrangement of the gene and seems to be secondary to transcriptional activation of the message.

lck mRNA is transcribed from two promoters, yielding two transcripts differing only at their 5' untranslated termini (Garvin *et al.*, *MCB* 8:3058). Using nuclease protection mapping techniques, we find that in normal lymphoid cells, transcript 1, transcribed from the upstream start site, is at least fifty times more abundant than transcript 2. In lymphoid tumor cell lines, the second, downstream-initiated transcript, is usually much more abundant, in some cell lines approaching the levels of transcript 1. In contrast, transcript 1 is not detected in the non-lymphoid cell lines, in which only transcript 2 is present. We conclude that expression of *lck* transcribed from its upstream start site is lymphoid cell-specific, whereas expression of *lck* by non-lymphoid tumor cells is due to selective induction of transcription from its downstream promoter. Studies to determine the mechanism of this induction and its functional significance are underway.

E 235 THE EFFECTS OF MICROINJECTION OF ANTI-*SRC* ANTIBODIES INTO LIVING CELLS. Paul A. Walton♣, Joan Brugge♥, and Jim Feramisco♣. ♣ Dept. of Medicine, Univ. of California at San Diego 92093 and ♥ Dept. of Microbiology, S.U.N.Y., Stony Brook, NY. 11794. The protein products of the *v-src* and *c-src* genes are membrane-bound phosphoproteins with intrinsic tyrosine-specific protein kinase activity. pp60$^{v\text{-}src}$, the product of the viral oncogene, can transform cells by an as yet unknown mechanism. The function of pp60 $^{c\text{-}src}$, the cellular homolog, remains unknown. As part of a study to illucidate the effects of the *src* proteins on cell function and growth we designed experiments employing the microinjection of monoclonal antibodies to *src* (327) into tsLA90-3T3 cells. These cells express a temperature-sensitive mutant of *v-src*. At the non-permissive temperature (41°C), the expressed v-*src* protein is bound in an inactive form with the 90 kD heat-shock protein. After shifting down to the permissive temperature (37°C), the v-*src* is released to the membranes and the cells express the transformed phenotype. Cells were preincubated in low serum (0.1%) at 41°C for 24 hours, to synchronize the cells at G°. Microinjection of antibodies was then performed using microcapillary needles. The cells were incubated at 37°C for 24 hours in the presence of ^{3}H-thymidine. Following this incubation, the cells were fixed, permeablized, and immunostained for the presence of mouse antibodies using Rhodamine-conjugated goat-anti-mouse antibodies. After immunostaining, the cells were overlayed with nuclear track emulsion, exposed for four days, and developed. By comparing the extent of ^{3}H-thymidine incorporation between cells that were positive for the presence of mouse anti-src antibodies with control cells, it was observed that fewer injected cells incorporated ^{3}H-thymidine than did control cells. The cells were indistinquishable in morphological aspects. These results demonstrate that the anti-src antibodies can delay or prevent the entry into the S phase of the cell cycle. We now intend to employ these antibodies as reagents along with microinjected *v-src* protein to study the events that link the appearance of this protein with the events of cell transformation.

E 236 PDGF-RECEPTOR TYPE B IN HUMAN MENINGIOMA CELL CULTURES, Jia-Lun Wang, Monica Nistér, Bengt Westermark and Jan Pontén, Department of Pathology, University Hospital, S-751 85 Uppsala, Sweden. Human meningioma is a mostly benign tumor derived from arachnoid tissues. We have investigated the presence of functionally active PDGF-receptors on human meningioma cells in culture. Tumor samples were obtained from three surgically removed meningiomas and normal arachnoid tissue from an autopsy case. Cells were routinely cultured in Eagle's MEM 10% FCS. Effects of PDGF-AA and BB dimers on DNA synthesis were measured as 3H-thymidine incorporation during 48 h of labeling cells maintained in Eagle's MEM 0.5% FCS. PDGF-BB but not AA stimulated DNA synthesis in all three tumor cell cultures. Addition of 20ng/ml PDGF-BB increased 3H-thymidine incorporation to about 1.5-2.0 times backgroud values. Binding studies were performed by using 125I-labeled recombinant PDGF-AA and PDGF-BB. Only 125I-PDGF-BB showed a specific binding to all tumor cell cultures after incubation of cells for 2 h at 4°C. Total cellular RNA was analyzed by Northern blotting and hybridization with a 32P-labeled human PDGF-receptor type B probe. The data confirmed the presence of PDGF-receptor type B RNA in both the tumor cell and arachnoid cell cultures. The present study shows that meningioma and arachnoid cell cultures have PDGF-receptors mainly of type B, although a low amount of PDGF A receptor binding sites cannot be excluded.

E 237 CLONING OF THE MURINE C-FGR PROTO-ONCOGENE AND REGULATION OF ITS EXPRESSION IN MONOCYTIC CELLS, Cheryl L. Willman, Yi Tao-lin, and Jeffrey W. Potter, Dept. of Cell Biology, University of New Mexico School of Medicine, Albuquerque, NM 87131.
While all of the src-related tyrosine kinases have highly conserved carboxy terminal catalytic domains, their unique N terminal domains may mediate substrate specificity, intracellular location, and lineage of expression. We have determined that normal bone marrow-derived monocytic cells express three of these kinases: fgr, hck, and lyn. In contrast to the constitutive expression of hck and lyn, c-fgr is transiently induced by monocytic growth factors (CSF-1), as well as by factors which activate monocytes to functional states (GM-CSF, LPS, and interferon gamma). Maximal levels of c-fgr transcripts are induced 8 hours after the addition of each factor and diminish by 24 hours; c-fgr mRNA induction is post-transcriptional and inhibited by cycloheximide. While lyn transcripts are not modulated by these factors, hck levels are transiently elevated only by LPS. These data imply that these kinases have distinct functions in monocytes. Full length c-fgr clones were isolated by constructing a lambda gt10 cDNA library from murine monocytic cells stimulated for 8 hrs by CSF-1. A 2.2kb clone contained a 1551 base coding sequence and 5' and 3' untranslated regions of 185 and 428 bases, respectively. Sequence analysis revealed that this clone had highest homology to v-fgr; however our murine c-fgr cDNA clone has a significantly different predicted amino acid sequence from residues 26-60 when compared to human c-fgr clones isolated from EBV transformed cells. The 3' untranslated region of c-fgr contains the conserved AU sequence mediating mRNA instability which may explain the transient expression of this gene compared to murine lyn and hck which lack this sequence. Analysis of kinase expression in myeloid stem cells reveals that c-fgr is not expressed until commitment to the monocytic lineage. We have constructed retroviral vectors expressing c-fgr and are examining the role of this gene in monopoiesis.

E 238 PREPARATION OF HIGH-AFFINITY MONOSPECIFIC ANTI-RB ANTIBODY AND ITS USE IN THE STUDY OF RB PROTEINS Hong-Ji Xu, Shi-Xue Hu, Tomoko Hashimoto, Rei Takahashi, Lucetta Caston, and William F. Benedict, Center for Biotechnology, Baylor College of Medicine, 4000 Research Forest Drive, The Woodlands, Texas 77381
The retinoblastoma susceptibility gene (RB) has now been identified. Some authors described a nuclear phosphoprotein which was detected by monoclonal and/or polyclonal antibodies against different epitopes of the putative RB gene product, while the biochemical activity responsible for the anti-oncogenic effect of RB proteins remains to be elucidated. We have, proceeding along this line, raised high titer antisera in pasteurella-free New Zealand White Rabbits (male) with mixed synthetic peptides corresponding to 3 epitopes of the deduced RB protein. Starting with these rabbit antisera, we applied a multistage purification to isolate high-affinity monospecific anti-RB IgGs, which combine certain major advantages of polyclonal and monoclonal antibodies. These pure IgGs are good for detecting a trace of RB proteins in cell lysates by immunoprecipitation, Western immunoblotting and high-resolution two-dimensional polyacrylamide gel electrophoresis. They also allow RB proteins to be concentrated from normal human cells by immunoaffinity chromatography. Our preliminary data shows that the purified high-affinity anti-RB IgGs immunoprecipitated at least two protein doublets. These isoantigenic proteins are present in human normal fibroblast cell lines, e.g., WI38 and WS1, but are not detectable in retinoblastoma cell lines examined. RB proteins from some non-retinoblastoma tumor cell lines might have different isoantigenic protein patterns and this work is still in progress.

E 239 EFFECTS OF PROTEIN KINASE C (PKC) DEPLETION ON EPIDERMAL GROWTH FACTOR RECEPTOR (EGFr) SIGNAL TRANSDUCTION, Peter P. Yu and John Mendelsohn, Department of Medicine, Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, NY 10021. In order to study the role of protein kinase C in EGF signal transduction, we depleted A431 cells of PKC by prolonged exposure to 12-O-tetradecanoylphorbol-13-acetate (TPA) 150 nM for 24h. Depletion of PKC was demonstrated by loss of Ca/phospholipid stimulation of histone phosphorylating activity, and by reappearance of the high affinity EGFr, which is eliminated after exposure to TPA for short intervals. Under conditions of PKC depletion, EGF induction of EGFr tyrosine kinase activity was increased, as measured by EGFr autophosphorylation. This finding is consistent with PKC serving as a negative feedback signal for EGFr tyrosine kinase activity. Despite the increase in tyrosine kinase signal, EGF induction of c-myc and c-fos mRNA transcription was reduced by PKC depletion. This indicates that PKC has a role as a second messenger in EGF regulation of nuclear events such as oncogene expression. Such a role is specific to certain messages, since PKC depletion resulted in greater induction of TGF alpha mRNA expression by EGF. Thus PKC depletion is associated with an increase both in ligand transcription and in ligand-inducable tyrosine kinase activity. Despite this, there was no enhancement of EGF effects on A431 cell growth.

E 240 SPONTANEOUS TRANSFORMATION OF DERMAL FIBROBLASTS FROM LI-FRAUMENI CANCER PATIENTS, Farideh Zamaniyan, Sun O. Yim and Michael A. Tainsky, Dept. of Tumor Biology, M.D. Anderson Hospital Cancer Center, Houston, Texas 77030

Families with the Li-Fraumeni syndrome show an inherited pattern of sarcomas and various other types of cancer that follow a dominant mode of transmission, have an early age of onset and exhibit multiple primary tumors. As soft tissue sarcomas including fibrosarcomas are frequently observed in this syndrome, fibroblasts offer an "at risk" target tissue to observe phenotypic characteristics of this unusual syndrome. We therefore investigated the in vitro growth characteristics of fibroblasts derived from skin biopsies of Li-Fraumeni syndrome cases and controls. Control fibroblasts maintain a normal morphology and eventually senesce in culture. Fibroblasts from affected individuals develop changes in morphology, chromosomal abnormalities, a growth crisis during which they begin to senesce but then recover. The cells then grow rapidly and maintain the morphology of a transformed cell. Thus, the spontaneous transformation of skin fibroblasts, an extremely rare event in cells derived from normal donors, occurs frequently in cells derived from Li-Fraumeni patients and may have predictive value for the determination of gene carriers at high risk of cancer. The transformed fibroblasts exhibit anchorage independent growth, secrete growth factors but are nontumorigenic in athymic nude mice. We have also observed that these transformed fibroblasts can be induced to form tumors in nude mice by an activated H-ras oncogene. These cells therefore undergo many steps associated with carcinogenesis and provide a useful model for the role of oncogene and growth factors in tumorigenesis.

Differentiation; Growth Factors and their Receptors-I

E 300 **Enhanced *jun* gene expression is an early genomic response to transforming growth factor-β stimulation**

Kari Alitalo, Liisa Pertovaara, Lea Sistonen, Timothy Bos, Peter Vogt and Jorma Keski-Oja Department of Virology, University of Helsinki, 00290 Helsinki 29, Finland and Department of Microbiology, University of Southern California, Los Angeles CA 90033-1054

TGFß is a multifunctional polypeptide that regulates proliferation, differentiation and other functions of many cell types. The pathway of TGFß signal transduction in cells is unknown, but it may involve a delayed breakdown of inositol phospholipids. Previous studies have shown that the transcription of the c-*fos* oncogene is markedly elevated within four hours after the adminstration of TGFß to sensitive cells. We find that an early effect of TGFß is an enhancement of the expression of the *jun*B gene and the c-*jun* proto-oncogene at the mRNA and protein level. The stimulation can be seen in both human lung adenocarcinoma A549 cells which are growth-inhibited by TGFß , in AKR-2B mouse embryo fibroblasts which are growth-stimulated by TGFß and in K562 human erythroleukemia cells, which are not appreciably affected in their growth by TGFß. The increase of *jun*B and c-*jun* mRNA occurs with picomolar TGFß concentrations; however, differential and cell-specific regulation determines the exact timing and magnitude of the response of each *jun* gene in a given cell. Thus one of the earliest genomic responses to TGFß may involve nuclear signal transduction and amplification by the c-fos, *jun*B and c-*jun* transcription factors. The differential activation the *jun* genes may explain some of the pleiotropic effects of TGFß.

E 301 EXPRESSION OF MITOGENIC AND ANGIOGENIC GROWTH FACTOR GENES IN PRIMARY HUMAN ASTROCYTOMAS, M. Maxwell, S. Naber, H. Wolf, T. Galanopoulos, P. McL. Black, H.N. Antoniades, The Center for Blood Research, Department of Neurosurgery, Brigham & Women's Hospital, Harvard Medical School, and the Department of Pathology, New England Medical Center, Boston, MA 02115

PDGF has recently been implicated in normal gliogenesis of the central nervous system by controlling the differentiation of glial cells. Since progressive de-differentiation with increasing malignancy is characteristic of human gliomas, we have examined 40 primary human gliomas for the expression of PDGF-1, c-*sis*/PDGF-2, PDGF-Receptor (PDGF-R) genes, and glial fibrillary acidic protein (GFAP), an index of differentiation and anaplasia of glial neoplasms. Over 80% of high grade gliomas were found to express both c-*sis*/PDGF-2 and PDGF-R genes. PDGF-1 chain gene was expressed at lower levels in only 35% of gliomas. A characteristc of the glioblastoma multiforme, the most malignant human brain tumor, is the presence of vascular hyperplasia within the neoplasm. We have studied the expression of the angiogenic growth factors acidic fibroblast growth factor (aFGF) or endothelial cell growth factor (ECGF) and transforming growth factor-alpha (TGFα) in 40 high grade primary human astrocytomas. ECGF is highly expressed in over 70% of the tumors, a similar pattern is also found in the case of TGF-α. These findings are confirmed by Western blot analysis and *in situ* hybridization.

E 302 CANCER-RELATED PROTO-ONCOGENES ARE EXPRESSED IN NON-MALIGNANT HUMAN BREAST TISSUE, Sue A. Bartow and Richard T. Hildebrandt, Department of Pathology, University of New Mexico School of Medicine, Albuquerque, NM 87131.
Increased epidermal growth factor receptor expression in human breast cancer has been found to be correlated with progression of disease. The c-erb b 2 (HER-2 neu) proto-oncogene is closely related to the epidermal growth factor gene, and its amplification and increased expression in human breast cancer has also been associated with aggressive disease. The int-1 and int-2 proto-oncogenes are activated by proviral insertion of mouse mammary tumor virus, and induced expression of these genes is associated with breast carcinoma in the mouse. Recent data suggest that the int-2 gene encodes a fibroblast-like growth factor. Amplification of the int-2 gene has been described in human breast carcinomas. We have identified a 4.8 kb transcript of the c-erb b 2 gene and a 3.2 kb transcript of the c-int-2 gene in non-malignant pubescent and adult luteal phase human breast tissue. To date we have detected no int-1 transcripts in these breast tissues. These findings further implicate the presence of critical periods of gene activation during which the human breast is more likely to be sensitive to initiation of carcinogenesis.

E 303 THE EXPRESSION OF EGF RECEPTOR AND EGF RELATED PEPTIDES IN OVARIAN AND CERVICAL CARCINOMAS, Thomas Bauknecht, Manuela Kohler, Dietmar Schwörer. Universitäts-Frauenklinik, 78 Freiburg, Hugstetterstr. 55, FRG. We reported earlier that EGF-R are detected by binding studies in 35% of ovarian and >90% of cervical carcinomas. We described also the existence of EGF related growth factors in the extracts of these tumors. In this study the number of EGF-R binding sites in ovarian and cervical carcinomas were compared with the results of immunohistochemistry and northern blot analysis. The EGF related growth factors were further characterized by a TGFa Ria. The immunohistochemistry showed that only the tumor cells are EGF-R^+, stromal cells were EGF-R^-. The highest EGF-R staining reaction were found in squamous cell carcinomas. Cells of squamous cells carcinomas which have reached terminal differentiation stained negatively. In ovarian carcinomas EGF-R^+ tumors were composed of EGF-R^+ and EGF-R^- clones. Biochemically EGF-R^- ovarian carcinomas were also immunohistochemically EGF-R^-. The analysis of EGF-R specific mRNA revealed a close correlation of the amount of mRNA and EGF-R binding data. No significant correlation were found between the amount of EGF-R mRNA and myc mRNA. By the use of a TGFa specific Ria different concentration of TGFa were found in tumor extracts but also in premenopausal ovaries. The analysis of TGFa specific mRNA in the same tissues is still under investigation. By the use of southern blotting we did not observe EGF-R or TGFa gene amplification in tumors with enhanced EGF-R or TGFa expression. From this we conclude that cis or trans acting factors influence the expression of the EGF system stimulating the tumor progression.

E 304 RESPONSE OF PROTEIN KINASE C (PKC) ACTIVITY TO GASTRIN-RELEASING PEPTIDE (GRP) IN HUMAN SMALL CELL LUNG CARCINOMA (SCLC) NCI-H345 CELLS, Larry Beck, John Brozna, Lynda Brown, and Madeleine Kane, Medical Oncology Division, Denver VAMC, Denver, CO 80220 H345 SCLC cells utilize GRP in an autocrine growth loop. While PKC plays a critical role in mitogenesis stimulated by GRP-like peptides in mouse fibroblasts, its role and response to pharmacologic manipulation has not been described in human SCLC cells. To study this we have adapted an assay for PKC enzymatic activity to H345 cells by defining optimal or acceptable constant conditions for cell density, substrates, co-enzyme concentrations, and time: e.g. cell density, $4x10^5$ cells/ml; histone III-S, 0.16 mg/ml; diacylglycerol, 2 ug/ml; phosphatidylserine, 20 ug/ml; calcium, 0.6 mM; ATP, 0.72 mM. Under these conditions, the time course of PKC activity was linear for at least 20 min. for both soluble cytosol (S_1) and triton solublized membrane (S_2) fractions. When intact H345 cells were preincubated at 37 C under various conditions, phorbol-12-myristate-13-acetate (PMA) but not phorbol-20-oxodeoxy-12,13-dibutyrate caused persistant decrease in S_1 PKC activity to 17% of control (203 pmol ^{32}P/min/10^6 viable cells) as expected. Preincubation for 10 minutes with GRP_{14-27}, 5nM, transiently decreased PKC activity to 26% of control activity. These results suggest that PKC activity in human SCLC is GRP responsive and may in part mediate the mitogenic effects of GRP.

E 305 DELETION ANALYSIS OF THE HUMAN EGF RECEPTOR: THE C-TERMINUS FACILITATES CELL PROLIFERATION AND TRANSFORMATION. Laura Beguinot[1], Thierry Velu[2] and Doug Lowy[2]. [1]University Institute of Microbiology, Copenhagen, Denmark [2]Laboratory of Cellular Oncology, NIH, Bethesda, Md.

We have previously reported that retroviral vectors containing the human EGF receptor cDNA (hEGFR) can induce high expression of normal human receptors and ligand-dependent transformation of NIH3T3 cells. To further examine EGFR physiology, we have now studied the relationship between transforming ability and biologic function of the EGFR C-terminus. We have constructed two premature termination mutants, Dc19 and Dc63, deleting the C-terminal 19 or 63 aminoacids from the full length (f.l.)EGFR. These mutations eliminate one or two C-terminal tyrosine residues (tyr 1173 and 1148); similar deletions are found in v-ErbB. These mutants confer EGF-dependent focal transformation and anchorage indipendent growth of NIH3T3 cells, however with lower efficiency than the f.l. receptor. A low number of foci and colonies in agar are observed with Dc63 (5 fold less) while Dc19 gives intermediate numbers (2fold less). Mutant EGFRs are expressed at the same level and about as stable as f.l.EGFR. Similarly EGF binding, EGF-dependent internalization and autophosporylation are not affected by the deletions. Therefore the 63 C-terminal aminoacids have no detectable influence on EGF-induced early events. Deletion of all the three autophosphorylation sites (tyr 1173,1148,1068) have then been tested and show an even lower transforming activity. We conclude that the EGFR C-terminus does not have a negative function, rather it may facilitate EGF-dependent cell proliferation.

E 306 IDENTIFICATION AND REGULATION OF S6 PROTEIN KINASE II IN MITOGEN-STIMULATED ANIMAL CELLS, J. Blenis*, R.L. Erikson‡, R.-H. Chen*, L.J. Sweet‡ and D.A. Alcorta‡, *Department of Molecular Biology, Northwestern University Medical School, Chicago, IL 60611 and ‡Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138

Antiserum to recombinant avian and Xenopus S6 kinase II (S6KII) have been used in combination with an immunocomplex-S6KII protein kinase assay to identify and characterize the regulation of S6KII activity in a variety of cultured animal cells. Analysis of S6KII immunoprecipitated from quiescent avian, murine or human cells by SDS-PAGE reveals the presence of several polypeptides with apparent M_r ~ 78-88 kDa. Within minutes of growth factor addition the mobility of several of these polypeptides changes, consistent with protein phosphorylation. Indeed, maximal phosphorylation is observed within 5 to 10 minutes after serum addition and this change in phosphorylation correlates with the rapid increase in S6KII-immunocomplex kinase activity. These results suggest the potential existence of a family of S6KII polypeptides and/or multiple S6KII phosphorylations. Consistent with regulation of S6KII by protein phosphorylation, we have identified a mitogen-stimulated S6 protein kinase II-protein kinase activity. The regulation of S6KII activity and its role in cell proliferation is being further examined in hopes of improving our understanding of signal transduction and cell growth.

E 307 CHARACTERIZATION OF THE MET ONCOGENE AND PROTO-ONCOGENE PRODUCTS AND TRANSFORMING ACTIVITIES. M. Bodescot[1], M.I. Gonzatti[1], T. E. Kmiecik[1], M. Park[1], A. Iyer[1], S. Showalter[2], D. Blair[3] and G.F. Vande Woude[1]; [1]BRI-Basic Research Program; [2]Program Resources, Inc.; [3]Laboratory of Molecular Oncology, NCI-Frederick Cancer Research Facility, Frederick, Maryland 21701, USA.

The MET proto-oncogene is a member of the trans-membrane tyrosine-kinase growth-factor receptor gene family. The primary translation product (p150) is glycosylated to produce p170, which is cleaved to yield p140 and p30. The MET oncogene contains the region coding for the kinase domain fused to unrelated 5′ sequences (TPR). Characterization of cDNAs indicates that the MET oncogene encodes two proteins as a result of alternative splicing within the TPR region. The first protein contains 76 additional N-terminal amino-acids, as compared to the second one. Both proteins exhibit *in vitro* kinase activity and transforming activity.

The mouse MET proto-oncogene cDNA placed under the transcriptional control of the Moloney MSV LTR transforms mouse NIH 3T3 cells upon transfection, whereas the human counterpart does not. The use of chimeras of the two cDNAs indicates that the mouse extracellular domain is necessary for transformation. Since human p170 is not processed in mouse NIH 3T3 cells, this suggests that proteolytic cleavage is necessary for transforming activity.

Finally, the transforming potential of the human MET proto-oncogene cDNA can be activated by deletion of both a part of the extracellular domain and the C-terminal region, which includes putative regulatory tyrosine residues.

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E 308 EFFECTS OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR (GM-CSF) AND DIMETHYL SULFOXIDE (DMSO) ON THE CELL CYCLE PROGRESSION AND PHOSPHOPROTEINS OF G1-SYNCHRONIZED HL-60 CELLS. JK Brennan, KS Lee, J Amos, CN Abboud, and PC Keng, University of Rochester School of Medicine, Rochester, NY. In previous studies of asynchronously growing HL-60 cells, we found that GM-CSF restored growth in DMSO-inhibited cultures without impairing DMSO-induced maturation - i.e., resulted in a facsimile of normal development where renewal and maturation are balanced. We have now studied the effects of DMSO/GM-CSF on the cell cycle movement and phosphoproteins of highly enriched populations of G1 cells obtained by centrifugal elutriation. Cycle kinetics were followed by changes in cell volumes, cell concentrations, and by flow quantitation of DNA/RNA content following staining with mithramycin or acridine orange. Phosphoproteins were analyzed on autoradiograms of [^{32}P]-orthophosphate-labeled cell lysates subjected to giant 2-D gel electrophoresis. GM-CSF accelerated, and DMSO inhibited G1 to S progression - especially in the 2nd cycle following synchronization. Cultures with both GM-CSF and DMSO had intermediate values. Parallel interactions of GM-CSF and DMSO were noted on the phosphorylation of two proteins (Mr 60 kDa, pI 5.6; 50 kDa, pI 5.1) after only 15 minutes of culture when the cells were in early G1. These findings suggest that the opposing effects of GM-CSF and DMSO on G1 to S transit may relect antagonistic effects on protein phosphorylation occurring well in advance of the time when cell cycle progression is affected.

E 309 **Alteration of IGF expression in vivo and in vitro in human breast cancer.** Nils Brunner, Kevin J. Cullen, Robert Clarke, Doug Yee, Neal Rosen, Francis G. Kern, and Marc E. Lippman. Lombardi Cancer Center, Georgetown Univ., Washington, D.C. 20007. We have previously shown that IGF-I and IGF-II mRNAs are easily detected in most human breast tumor specimens. No breast tumor epithelial lines express authentic IGF-I mRNA, and only MCF-7 and T47-D cells express IGF-II mRNA. In T47-D cells, IGF-II expression is estrogen regulated. Additionally, IGF-I and IGF-II are potent mitogens for breast tumor epithelial cells. Using an RNAse protection assay with human riboprobes, we examined breast tumor lines for differences in expression of IGF I and II mRNA in tumor cells grown either in vitro or in vivo in ovariectomized nude mice. No IGF-I mRNA was seen in any specimens. In ER negative MDA MB-231 and MDA MB-468 cells, IGF-II message was not detected in cells growing in vitro, but was easily seen in tumors grown in nude mice. The human IGF riboprobes did not detect mouse IGF species, so the message detected was of human origin. In addition we analyzed M-III, a tumor line derived from estrogen-dependent MCF-7 cells which does not require estrogen for tumor formation or growth but which retains estrogen receptor and some estrogen response in vivo. These cells showed little IGF-II mRNA expression in vitro with or without E2. Tumors grown in vivo without E2 also had little detectable IGF-II mRNA. However, M-III tumors grown in nude mice with estrogen showed marked increase in expression of IGF-II mRNA. We conclude that host factors not duplicated in cell culture systems are important in the regulation of of IGF expression in vivo. In addition, alterations in the regulation of IGF expression by estrogen may accompany the transition from estrogen dependence to estrogen independence.

E 310 GROWTH FACTOR CONTENT IN BREAST CANCER BIOPSIES, V. Cappelletti, P. Miodini, D. Coradini, C. Ruedl and G. DiFronzo, Instituto Nazionale Tumori, Milano, Italy
We have previously demonstrated the existence of an inverse correlation between EGF-r and steroid receptor content in human breast cancer biopsies. In breast cancer cell lines EGF-r appears to be down regulated following the interaction with its ligand (either EGF or TGFa); it appeared therefore interesting to investigate the relationship between the EGF-r concentration on the cellular membrane and the growth factor content in breast cancer solid tumors too. Accurate measurement of the growth factor content in solid tumors was hindered by the difficulty in obtaining high-yield growth factors preparations from neoplastic tissue due to the action proteolitic activities and to segregation by binding proteins. Further, most methods require an acid extraction step which is likely to activate high molecular weight precursors causing problems for a correct interpretation of results. This study was mainly focused on EGF, TGFa and other, not yet well defined peptides, able to interact with the EGF-r. Human breast cancer biopsies were processed for estimation of growth factor content using the acid/ethanol extraction procedure, the acid/acetone protocol and a direct assay on acid/ethanol treated cytosol. The extracts obtained by the various methods were then analyzed by radioreceptor assay and radioimmunoassay. When enough tissue was available, EGF-r and steroid receptor were determined on the same biopsy. EGF-r and growth factor content appeared to behave as independent variables. Implications of these findings and relative merits and liabilities of the various extraction procedures will be discussed.

E 311 CONDITIONAL TRANSFORMATION BY v-sis IN NIH-3T3 CELLS, Philip M. Carpenter, Mark K. Mercola, Daniel A. Mercola, Department of Pathology, UCSD, La Jolla, CA 92093 and the Veterans Administration Medical Center, San Diego, CA 92161 and Dana-Farber Cancer Institute, Boston, MA 02115.

The v-sis gene and its cellular homologue, the B chain of PDGF are thought to transform by autocrine stimulation of PDGF receptors. We have observed (1,2) that sis activated genes such as c-fos may participate in this mechanism. To test this hypothesis, plasmids containing the metallothionein promoter and the v-sis gene in either the sense (pMTsis) or the antisense orientation (pMTsas) have been prepared. In a focus forming assay, pMTsis produced an average of 177 transformed foci compared to 11 without zinc and pMTsas produced 25 foci in the presence of zinc compared to 12 without zinc. Random bovine carrier DNA alone resulted in 23 foci. Furthermore, pMTsis and pMTsas containing clones, isolated by cotransfecting cells with a neomycin resistance plasmid, show average doubling times of 30 and 72 hours respectively. We have now isolated 12 sense and 5 antisense clones. All of the sense clones exhibit prominent morphological features of transformation such as spontaneous focus formation, while antisense clones grow as flat sheets. We conclude that these clones exhibit conditional and potentially reversible transformation and may be useful in the analysis of secondary gene activation. Molecular analysis is underway to confirm these results. 1. Mercola, D., et al., (1987) BBRC, 147, 288; 2. Mercola D., et al., (1988) Gene, (in press).

E 312 INTERLEUKIN 2 ACTIVATES A TYROSINE PROTEIN KINASE THROUGH THE 75KD RECEPTOR ON HUMAN LEUKEMIA CELL LINES, John E. Casnellie & Elizabeth M. Saltzman, Department of Pharmacology and Cancer Center, University of Rochester Medical Center, Rochester, N.Y. 14642. We have previously shown that interleukin 2 (IL-2) stimulation of T cells results in an increase of tyrosine phosphorylations of several proteins (J. Biol. Chem.(1988) 263, 6956) The phosphorylations were observed in IL-2-dependent cells at concentrations of IL-2 specific for binding to the high-affinity receptor. The high-affinity receptor for IL-2 consists of two different IL-2-binding proteins with molecular weights of 55,000 (p55) and 75,000 (p75). The p75 receptor, independent of p55, appears to be responsible for IL-2-induced signal transduction that results in proliferation. If activation of a tyrosine protein kinase is essential to the mechanism of IL-2-induced signal transduction then the p75 receptor alone would expected to activate a tyrosine protein kinase in response to IL-2-stimulation. We assessed the ability of IL-2 to induce tyrosine protein kinase activation via p75 by studying this response in the leukemic cell lines Hut 78 and YT. These cells have been found to express p75 as the predominant receptor for IL-2. Treatment of these cells with IL-2 resulted in an increase in tyrosine phosphorylation of proteins with molecular weights identical to that previously observed in IL-2-dependent cells where the signal transduction occurs through the high affinity-receptor. The concentrations of IL-2 required to induce these phosphorylations were the same as required for binding to the isolated p75 receptor. The phosphorylations were not affected by antibodies that inhibit binding of IL-2 to the p55. These results indicate that the signaling capacity for IL-2-induced increase in tyrosine protein kinase activity resides in the p75 receptor.

E 313 TGF-ß RECEPTOR PROTEOGLYCAN BINDS TGF-ß IN ABSENCE OF GAG CHAINS Sela Cheifetz and Joan Massagué, Department Biochemistry, University of Massachusetts Medical Center, Worcester, MA 01655

The high molecular weight Type III receptor for transforming growth factor ß (TGF-ß) is a membrane bound proteoglycan. Thus, incubating cells with glycosaminoglycan (GAG) degrading enzymes before or after affinity labeling with ^{125}I-TGF-ß converts theType III receptor (average M_r=280K) to smaller proteins of M_r=110K-130K. However, studies of these sorts do not address the question of whether or not GAG chains are required for expression of Type III receptor on cell surface or for generating the conformation necessary for ligand binding. The use of mutant CHO cell lines with defects in initiation or elongation of GAG chains permits a more direct examination of the role of GAG chains in defining the properties of the Type III TGF-ß receptor. As expected, the mutant cells lacked the high molecular weight affinity labeled type III receptor. Instead, these cells have two new proteins of M_r = 110K-130K consistent with the conclusion that the GAG chains are not required for ligand binding or expression of the receptor on the cell surface. Other characteristics under investigation are the stability,affinity and intracellular dynamics of mutant.relative to wild type receptors.

E 314 IDENTIFICATION AND CHARACTERIZATION OF THE BCL-2 PROTEIN – A CANDIDATE PROTO-ONCOGENE PRODUCT ASSOCIATED WITH t(14;18) TRANSLOCATIONS. Zehava Chen-Levy, Jamison Nourse and Michael L. Cleary, Department of Pathology, Stanford University Medical Center, Stanford, CA 94305-5324.

We have identified the human bcl-2 candidate proto-oncogene product and studied its biochemical properties. Using polyclonal antibodies, a single 24-kilodalton protein was detected in certain human lymphoid cell lines and tissues. The abundance of the protein correlated with bcl-2 RNA levels found in the same cell lines. Especially high levels of BCL-2 expression were observed in lymphoid cells carrying t(14;18) translocation.

Biochemical studies demonstrated that the bcl-2 protein has a lipophilic nature and is integrally associated with cellular membrane structures, probably by means of a hydrophobic carboxy terminal membrane spanning domain. Its subcellular localization, examined in t(14;18) carrying cells, is in the perinuclear endoplasmic reticulum and in the plasma membrane. The BCL-2 appears to be a non-glycosylated, single subunit, membrane spanning protein with a proposed topology distinct from previously described proto-oncogenic proteins.

E 315 **Type I and type II IGF receptor expression and function in human breast cancer.** Kevin J. Cullen, Doug Yee, James F. Perdue*, Brian Hampton*, William S. Sly*, Marc E. Lippman and Neal Rosen. Lombardi Cancer Center, Georgetown Univ., Washington, D.C. 20007. *Hammond Labs, American Red Cross, Rockville, MD. *St. Louis University, St. Louis, MO. We and others have previously reported that both IGF-I and IGF-II are potent mitogens for human breast cancer cells in culture. Additionally, we have shown IGF-I and IGF-II mRNAs are easily detected in the majority of breast tumor specimens examined, while no breast cancer epithelial cell lines express authentic IGF-I mRNA, and few lines express IGF-II mRNA. Using an RNAse protection assay, we examined breast tumor specimens and breast cancer epithelial cell lines for expression of mRNA for the type I and type II IGF receptors, as well as the insulin receptor. All of the specimens examined expressed mRNA for all three receptors. We then examined estrogen-dependent MCF-7 cells for the mitogenic effects of IGF-I and II in the presence of antibodies to both the type I and type II receptor. Alpha IR-3, a monoclonal antibody which blocks the type I receptor, abolished the mitogenic effects of both IGF-I and IGF-II. It did not, however, block the mitogenic effects of insulin. A polyclonal antibody which was able to partially inhibit binding of IGF-II to the type II receptor, did not alter the mitogenic effects of either IGF-I, IGF-II or insulin. Non specific control antibodies were also without effect. We conclude that type I and type II IGF receptors are ubiquitously expressed in breast cancer and our experiments with MCF-7 cells suggest the mitogenic effects of both IGF-I and IGF-II are mediated via the type I IGF receptor.

E 316 GROWTH FACTOR EXPRESSION AND SENSITIVITY OF HUMAN BREAST CANCER CELLS DURING THE PROGRESSION TOWARDS STEROID INDEPENDENCE, Roger J. Daly and Philippa D. Darbre, Cellular Endocrinology Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.

The human breast cancer cell lines T-47D and ZR-75-1 exhibit oestrogen responsive and oestrogen dependent growth in tissue culture, respectively. When maintained in the long term absence of steroid, both cell lines exhibit a loss of steroid sensitivity, such that although growth in the presence of oestrogen remains unaltered, there is a gradual increase in the growth rate in the absence of steroid. This provides a useful model system for tumour progression in vivo. In ZR-75-1 cells, these changes appear to be clonal, but occur at high frequency (approximately 1 in 1000 cells). After one year of steroid deprivation, ZR-75-1 cells exhibit an oestrogen responsive, rather than dependent, phenotype, whilst T-47-D cells have almost lost their growth response to the hormone. PS2 mRNA has been used as a molecular marker of oestrogen action in these cells. Continued sensitivity of this gene to oestradiol, after a year of steroid deprivation suggests that changes in cell growth occur without any loss of receptor function. In an attempt to understand the changes in growth control which occur as cells progress towards steroid independence, the sensitivity of the cells to serum and individual growth factors (TGF-α, TGF-β, insulin), and the expression of growth factor genes at both the mRNA and protein level, have been investigated.

E 317 **AN RNP AND A PROTEIN FACTOR INTERACT WITH A NUCLEASE HYPERSENSITIVE C-MYC DNA ELEMENT: POSSIBLE REGULATORS OF THE C-MYC GENE**, Terri L. Davis, Anthony B. Firulli, and Alan J. Kinniburgh, Roswell Park Memorial Institute, 666 Elm St., Buffalo, NY 14263. Cis-acting DNA sequence elements and trans-acting factors have been shown to interact to regulate gene expression. We have located a cis-acting, positive regulatory DNA sequence element within the 5' flanking DNA of the c-myc gene (-125 bp). This DNA sequence element has a large purine-pyrimidine strand asymetry reminiscent of H-DNA. Like H-DNA, this element is sensitive to a single-strand nuclease in-vitro. A novel factor with the properties of a ribonucleoprotein particle (RNP) interacts with this DNA region. The interaction of the c-myc DNA sequence element and the RNP involves an RNA-DNA hybrid. In addition, a protein(s) factor binds to this DNA sequence element. The protein(s) and the RNP factors are present in nuclear extracts from cells actively transcribing the c-myc gene. They are diminished or undetectable in cultured cells induced to differentiate, where c-myc transcription is down regulated. These results implicate a protein(s) and RNP particle as positive transcriptional regulators of the c-myc gene.

E 318 INHIBITORY ROLE OF TGF-β ON PMA INDUCED TRANSFORMATION OF JB6 CELLS, Fabrizio De Benedetti[1], Lydia A. Falk[2], Connie R. Faltynek[3], Francis W. Ruscetti[2], Nancy H. Colburn[4] and Joost J. Oppenheim[2], [1]Laboratory of Biochemical Physiology, [2]Laboratory of Molecular Immunoregulation, [3]BCDP-Program Resources, Inc., [4]Laboratory of Viral Carcinogenesis, National Cancer Institute, FCRF, Frederick, MD 21701
JB6 is a nontumorigenic murine epidermal cell line which undergoes an irreversible phenotypic modification when treated with tumor promoters, such as PMA. It loses anchorage dependence, as measured by colony formation in soft agar, and becomes tumorigenic in nude mice. The induction of transformation by PMA is inhibited by the addition of a variety of antipromoters, such as glucocorticoids, retinoids, and superoxide dismutase. TGF-β is a multifunctional protein which has been shown to either inhibit or stimulate cell growth and cell differentiation. We tested the effect of purified TGF-β on the induction of transformation of JB6 cells (clone 415a) by PMA. When TGF-β was added together with PMA in a soft agar assay, we found that it was able to significantly decrease, in a dose dependent manner (at concentrations of 1 ng/ml to 10 ng/ml), the number of colonies induced by PMA. This effect was not due to nonspecific inhibition of growth, since TGF-β did not inhibit the growth rate of untreated and PMA treated adherent JB6 cells. Another well known antiproliferative agent, IFN α-β at concentration of 2.5 U/ml to 750 U/ml, had no significant effect on colony formation induced by PMA. Moreover, we found that TGF-β enhances the antipromoting activity of suboptimal concentrations of retinoic acid. Our data suggest that TGF-β may play a role in the regulation of transformation of JB6 cells.

E 319 HUMAN EPITHELIAL CELLS INDUCE HUMAN MELANOCYTE GROWTH IN VITRO BUT ONLY SKIN KERATYNOCYTES REGULATE ITS PROPER DIFFERENTIATION IN THE ABSENCE OF DERMIS, Michele De Luca*, Fiorella D'Anna**, Sergio Bondanza*, Adriano Tito Franzi** and Ranieri Cancedda*, *Istituto Nazionale per la Ricerca sul Cancro and Istituto di Oncologia Clinica e Sperimentale dell'Università di Genova, 16132 Genoa, Italy; **Istituto di Anatomia Umana Normale dell'Università di Genova, 16132 Genoa, Italy.
Human keratynocytes isolated from a skin biopsy and cultured in vitro reconstitute a stratified squamous epithelium suitable for grafting on burned patients. We show that melanocytes co-isolated from the skin biopsy proliferate in the same culture conditions that allow keratynocyte growth and maintain differentiated functions (i.e. synthesize melanin granules, are regularly interspersed in the basal layer of the cultured epidermis and transfer melanosomes in the cytoplasm of contigous keratinocytes). Isolated melanocytes in culture grow in the presence of specific growth factors with a division time (d.t.) of 4-10 days. We further show that i) human keratinocytes and oral epithelial cells possess strong and specific melanocyte growth stimulating activity (d.t.: 24 hours), ii) melanocyte growth is not autonomous but require close keratinocytes contact and is regulated to maintain a physiological melanocytes/keratinocytes ratio, iii) pure skin keratinocytes but not oral epithelial cells have all the information required for the proper physiological location and differentiation of melanocytes in the epidermis.

E 319A GROWTH REGULATION OF HUMAN BREAST CANCER, G. DiFronzo, V. Cappelletti, G.Granata, D. Coradini and R. Silvestrini, Instituto Nazionale Tumori, Milano, Italy
Human breast cancer biopsies as well as human breast cancer cell lines possess high affinity epidermal growth factor receptors (EGF-r) as well as steroid receptors. Since the clinical relevance of the EGF-r content of human breast tumors is not yet known, we investigated its distribution in a series of 136 unselected operable breast tumors in relation to 3 well known prognostic variables: the estrogen receptor (ER) the progesterone receptor (PgR) and proliferative activity evaluated in terms of thymidine labeling index (TLI). EGF binding activity consisted of a single class of high affinity binding sites (kd=0.55 nM) and ranged from 0 to 275 fmol/mgP. There was a statistically significant ($p<0.001$) inverse association between EGF-r and cytoplasmic ER (ERc). In fact two-thirds of the tumors were either ERc+EGF-r- or ERc-EGF-r+. The same type of association was found between EGF-r and either nuclear ER (ERn) and PgR. The EGF-r content was significantly higher ($P<0.001$) in the ERc- tumors (72.6+54.4) compared to the ERc+ ones (33.0 +37.4 fmol/mgP). Similarly the subset of PgR + tumors was characterized by lower EGF-r mean concentrations with respect to PgR- cases (35.4+54.4 fmol/mgP versus 63.8 +54.4 fmol/mg P). A statistically significant ($p<0.05$) association was observed between proliferative activity and EGF-r status; 66% of EGF-r positive tumors were characterized by high thymidine labelling index and 62% of tumors with low proliferative activity were EGF-r negative. Practical implications of these findings will be discussed.

E 320 HL-60 CELLS CONSTITUTIVELY RELEASE A POLYPEPTIDE, WHICH DISTURBS THE INTERACTION OF VARIOUS TARGET CELLS WITH THEIR EXTRACELLULAR MATRIX, Klaus Dittmann, Petro E. Petrides, Munich University School, Molecular Oncology Laboratory, Groβhadern Marchioninistr. 15, 8000 Munich 70, West Germany. Leukemia is characterised by the appearence of immature white blood cells in the peripheral circulation. It is not known, however, how immature blood cells are able to egress from the bone marrow. Cells egressing from bone marrow or evading a blood vessel wall have to penetrate a barrier of basement membranes. We have developed an in vitro system, which allows to investigate the interactions of transformed blood cells - possessing enhanced evading capacity - with cells manufacturing basement membranes. We cocultivated the human leukemic cell line HL-60 with cells encountered by the leukemic cells during their life cycle such as fibroblasts, endothelial or epithelial cells. We have identified an activity constitutively produced by HL-60 cells, which interferes with the interaction of anchorage dependent cells with their extracellular matrix. The production of the molecule is stage specific since induction of HL-60 cells to terminally differentiated monocytes leads to a cessation of the release of the activity. Because the activity of the factor is destroyed by incubation with chymotrypsin and other proteases, we conclude that the factor is a polypeptide. Characterization and purification experiments show that the factor has a molecular weight of about 40000 daltons. The inhibitory effect of EDTA (10^{-5}M) upon this activity leads to the conclusion, that the detachment mechanism at least could be mediated in part of a metalloprotease. The HL-60 derived factor could have important functions for the molecular mechanism of egression of these transformed cells from the bone marrow as well as their penetration of blood vessel walls. Supported by a grant from DFG (SFB 324)

E 321 RETINOIC ACID CAUSES A DECLINE IN TGF-α EXPRESSION, CLONABILITY, AND TUMORIGENICITY IN A HUMAN EMBRYONAL CANCER CELL LINE. Ethan Dmitrovsky, Denise Moy, Wilson Miller, Angeline Li, and Hideo Masui. Department of Medicine, Memorial Sloan Kettering Cancer Center, NY, NY 10021. The human teratocarcinoma cell NTera-2 cl. D1 (NT2/D1) is a cloned embryonal cell line that differentiates into a neuronal phenotype and other lineages after retinoic acid (RA) treatment. The differentiated NT2/D1 cells have reduced stage specific embryonic antigen expression (SSEA-3) compared to undifferentiated cells. In RA-treated NT2/D1 cells mRNAs for the homeotic genes Hu-1 and Hu-2 are also induced. We sought to determine whether RA-treatment of these cells was associated with specific changes in growth factor expression or proliferative potential. We observed in the Northern analysis of RA-treated NT2/D1 cells a marked down-regulation of TGF-α expression within 24 hours of exposure. Recovery of TGF-α expression did not occur by day 6 when Hu-1 and Hu-2 mRNAs appear. The decreased TGF-α expression in RA-treated NT2/D1 cells was associated with a decline in clonability using both limiting dilution and soft agar cloning assays and tumorigenicity after a dose-dependent subcutaneous injection of cells into nude mice. We conclude that TGF-α expression correlated with the state of RA induced differentiation and the loss of malignant potential in NT2/D1 embryonal cancer cells.

E 322 AUTONOMOUS GROWTH OF A HUMAN NEUROBLASTOMA CELL LINE IS MEDIATED BY INSULIN-LIKE GROWTH FACTOR II, Osama M. El-Badry, Joyce A. Romanus, Lee J. Helman, Mark J. Cooper, Matthew M. Rechler and Mark A. Israel, Pediatric Branch, NCI, National Institutes of Health, Bethesda MD 20892 and Endocrinology Branch, NIDDK, NIH, Bethesda MD 20892.

Endogenously synthesized insulin-like growth factor II (IGF-II) stimulates the growth of a human neuroblastoma cell line, SK-N-AS. SK-N-AS cells grown continuously in a mitogen-free culture medium synthesize and secrete IGF-II (54-57ng/ml), but not IGF-I. These cells also possess type I IGF receptors on their surface that bind both IGF-I and IGF-II. A monoclonal antibody that inhibits the binding of radiolabeled IGF-I to the type I IGF receptor totally inhibits exogenous IGF-I and IGF-II stimulated DNA synthesis in SK-N-AS cells and partially inhibits basal DNA synthesis. High levels of IGF-II mRNA were detected in 2/8 neuroblastomas, tumors of the adrenal medulla that occur in very young children, and in 8/8 pheochromocytomas, tumors of adults that also arise in the adrenal medulla; lower levels were present in normal adrenal medulla. These tumors also expressed mRNA for the type I IGF receptor. Altered regulation of this growth stimulatory pathway may play a role in the pathogenesis of these tumors of the adrenal medulla.

E 323 INFLUENCE OF THE MAMMARY GLAND ON THE GROWTH, METASTASIS, AND DIFFERENTIATION OF A MURINE MAMMARY CARCINOMA, Bruce E. Elliott[1], Marian Arnold[1], Lori Maxwell[1], Wei Z. Wei[2], and Fred R. Miller[2]. [1]Cancer Research Laboratories, Queen's University, Kingston, Ontario, Canada, K7L 3N6 & [2]Michigan Cancer Ctr., Detroit, MI, 48201, U.S.A. Our laboratory has developed a spontaneous murine mammary carcinoma (SP1) model for studies of the immunology and physiology of the host/tumor relationship. The SP1 tumor grows more aggressively at a 10-fold lower LTD_{50} in the mammary gland compared to the subcutaneous site, with clear evidence of pulmonary metastasis in may (approximately 35%) of the animals which developed intramammary tumors. Tumor cells recovered from pulmonary lung metastases showed an increased ability to metastasize when reinjected into either the subcutaneous or mammary sites. Increased expression of class I MHC antigens, as well as certain basal and luminal breast epithelial markers, was observed during growth in the mammary gland as early as 7 days post injection, but not in the subcutaneous site at least until 21 days. In contrast to primary intramammary tumors, metastatic nodules showed low levels of class I MHC antigens while maintaining significant expression of basal and luminal epithelial markers. Removal of host epithelium by cauterization of the mammary bud at 3 weeks had no effect on metastasis and expression of epithelial antigens. Karyotype analysis of primary and metastatic tumors indicated that strong selection of SP1 tumor subpopulations occurs during intramammary growth and metastasis. The above findings suggest that selection (or induction) of distinct metastatic tumor subsets occurs during growth in the mammary stroma (supported by NCI(C) and MRC).

E 324 A MONOCLONAL ANTIBODY DISTINGUISHES TYPES 2 AND 3 PHOSPHATIDYLINOSITOL KINASES FROM BOVINE BRAIN
Gerda Endemann, Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305

A mouse monoclonal antibody (4C5G) has been raised against type 2 phosphatidylinositol (PI) kinase from bovine brain. The activity of partially purified type 2 PI kinase in Triton X-100 is inhibited 89% by the addition of 2 μg/ml of 4C5G. This antibody also immunoprecipitates a small amount of the PI kinase activity in NP-40 lysates of rat basophilic leukemia cells and NIH 3T3 cells.

Type 2 PI kinase, which constitutes 43% of total brain activity, is stimulated by non-ionic detergents and inhibited by adenosine (Endemann, G., Dunn, S.N. and Cantley, L.C., (1987) Biochemistry 26, p.6845). This activity is also present in fibroblast lines and in erythrocyte ghosts. The remainder of PI kinase activity in bovine brain, type 3, is also stimulated by non-ionic detergents, but is resistant to inhibition by adenosine. Type 3 PI kinase is not inhibited by 2 μg/ml of 4C5G.

E 325 THE EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTORS IN EXPERIMENTAL AND HUMAN ORAL CARCINOGENESIS, Stephen M. Game, Andrea J. Stone, Isabel J. Crane, Crispian Scully and Stephen S. Prime, Department of Oral Medicine, Surgery and Pathology, University of Bristol, BS1 2LY, UK.

This study examines EGF receptor expression in cultured keratinocytes from human oral squamous cell carcinomas (SCC) and in a rat model of oral carcinogenesis. Oral carcinomas of the tongue and palate were induced in Spraque-Dawley rats using the carcinogen 0.5% (w/v) 4-Nitroquninoline N-oxide 3 times weekly for 9 months. Cell lines were grafted to athymic mice to determine the degree of differentiation following selection in vivo. Receptors were quantified using an EGF radioreceptor binding assay. In both rat and human cell lines, keratinocytes from normal oral tissue had low affinity EGF receptors. Malignant keratinocytes predominantly expressed both increased (human) and decreased (rat) numbers of EGF receptors per cell. Epithelial cells from well-differentiated carcinomas (characterised by the elaboration of keratin) had low and high affinity EGF receptors and keratinocytes from less differentiated tumours predominantly had high affinity EGF receptors. The pattern of EGF receptor expression in xenograft cell lines corresponded closely to that of the original keratinocyte cultures prior to transplantation. The results suggest a correlation between the pattern of EGF receptor expression and tumour differentiation.

This work was supported by the Cancer Research Campaign.

E 326 ESTABLISHMENT OF CONTINUOUS CULTURES OF T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA CELLS AT DIAGNOSIS, Ruth Gjerset, Alice Yu and Martin Haas, Cancer Center, Department of Pediatrics, and Department of Biology, University of California, San Diego, La Jolla, CA 92093.
We have devised methods facilitating the establishment of continuous cultures of leukemic cells from patients with acute lymphoblastic leukemia of T-cell type (T-ALL) at diagnosis. The characteristics of T-ALL (diagnosis) cells were compared to those of the patients at the time of diagnosis. Cultured T-ALL (diagnosis) cells (a) had doubling times of 24-48 hours; (b) were dependent for growth on IL-2; (c) were reverse transcriptase negative; (d) did not form colonies in methyl cellulose; and (e) were monoclonal for T-cell receptor β chain rearrangements. Three T-ALL cultures had a normal diploid karyotype, and one had a 6q⁻ deletion which was also present at the time of diagnosis. Phenotypically the cultures were similar to cells at diagnosis. Phenotypically the cultures were similar to cells at diagnosis. They were clonally related to the original patient sample from which they were derived. Morphologically the cells were lymphocytes with a convoluted nucleus. Our results show that T-ALL samples can be grown into long-term cultures with high frequency. The cultured T-ALL (diagnosis) cells closely resemble the leukemic cells at diagnosis, they are dependent on IL-2 and they represent nonprogressed T-ALL cells with a minimum of chromosomal aberrations. Success at culturing the monoclonal population of T-ALL cells from patients at diagnosis should enable us to study the mechanism by which abnormal clones of blasts are induced, their role in the early phases of the disease, and ultimately how they relate to the disease at relapse.

E 327 CHARACTERIZATION OF HUMAN ERYTHROCYTES PURIFIED PHOSPHATIDYLINOSITOL-4 KINASE. Andrea Graziani and Lewis C. Cantley, Department of Physiology, Tufts University School of Medicine, Boston, MA 02111.
The turnover of Phosphatidylinositol (PI) pathway, through the hydrolysis of Phosphatidylinositol-4,5-bisphosphate (PI-4,5-P_2), has been proposed to play a role in the transduction of mitogenic signals. However, evidence for a direct biochemical link is lacking. Still relatively little information is available on the key enzymes involved. In our laboratory, a novel, PDGF activated, PI pathway, leading to the formation of the novel phospholipids, Phosphatidylinositol-3-phosphate (PI-3-P) and Phosphatidylinositol-3,4-bisphosphate (PI-3,4-P_2) has been found. Thus at least two pathways leading to the formation of distinct polyphosphoinositides exist. We have purified a PI kinase activity from human red blood cells (RBC), originally called type II, which features a relatively high Km for ATP, is inhibited by adenosine and is activated by non ionic detergent at concentration above the cmc. The type II PI kinase activity has been extracted from the human RBC membranes with Triton X-100, chromatographed first on anion exchange and one cation exchange in presence of non ionic detergent above the cmc, and then on an anion exchange column. A 56,000 dalton band has been identified as the type II PI kinase, by renaturation of the activity after SDS-PAGE electrophoresis. The purified PI kinase phosphorylates PI specifically and only on the D-4 position of the inositol ring, forming only PI-4-P as product. We also tested the ability of this enzyme to phosphorylate the D-4 position of PI-3-P to produce PI-3,4-P_2. This enzyme was at least 100 fold less active at phosphorylating this substrate compared to PI, suggesting that the PI-3,4-P_2 found in cells is not due to action of the PI-4 kinase on PI-3-P.

E 328 PRODUCTION OF IL-1 AND IL-6 LIKE FACTORS BY A HUMAN MEDULLOBLASTOMA CELL LINE. William N. Harrington, M.D. and Roberta L. Hayes, Ph.D., New York University School of Medicine, Depts. of Pathology and Neurosurgery, New York, NY 10016. Conditioned medium from TE671, a cell line derived from a human medulloblastoma was found to contain growth stimulatory activity for the Interleukin-dependent, murine T-cell clone D10.G4.1, similar to that of recombinant human IL-1 alpha. [Medulloblastoma is a type of primitive pediatric neuroectodermal CNS tumor, (PNET), which often has the ability to differentiate into glial or neuronal elements.] The IL-1-like activity from TE671 co-eluted with an autocrine growth stimulatory activity in the 12-29k dalton fraction by Sephacryl S200 gel filtration. Neither recombinant human IL-1a, IL-1 beta, nor IL-6, reproduces the autocrine growth stimulatory activity. However, immunocytochemically, TE671 cells produce molecules that are antigenically related to both IL-1 and IL-6. Moreover, RNA from TE671 cells hybridizes with cDNA probes to human IL-1B and murine IL-1a as well as IL-6, by dot blot analysis. The role of these cytokines in the proliferation and/or differentiation of malignant medulloblastoma cells and their normal counterparts remains to be defined.

E 329 HBGF-1 (aFGF) EXPRESSION In DDT1 AND LNCAP CELLS AND ISOLATION AND SEQUENCE ANALYSIS OF THE HBGF-1 GENE FROM A DDT1 COSMID LIBRARY, Stephen E. Harris°, Jeffrey A. Hall°, Hua Zhou°, Marie A. Harris° and Sheila M. Judge+, °W. Alton Jones Cell Science Center, Lake Placid, NY and +Department of Surgery, University of Chicago, Chicago, IL Selected clones of hamster DDT1 and human LNCAP cells are dependent upon androgen for growth. DDT1 tumor cells are derived from the smooth muscle layer of the ductus deferens. LNCAP cells are derived from a human lymph node carcinoma of the prostate. HBGF-1 (aFGF) or HBGF-2 (bFGF) can replace testosterone (T) in stimulating growth of the DDT1 cells, and this is correlated with the ability of testosterone to stimulate HBGF-1 mRNA 3-4x. Glucocorticoids inhibit this T induction of HBGF-1 mRNA. Similar experiments with LNCAP cells also indicate the HBGF family of growth factors can replace T in stimulating growth. The HBGF-1 mRNA levels are presently being analyzed in the LNCAP system. In order to understand the mechanisms of steroid regulation of HBGF-1 mRNA, the HBGF-1 gene was isolated from a DDT1 cosmids library. A 38 Kb DNA insert contains the entire transcription unit and ~7 Kb of 5'-flanking region. DNA sequence analysis and construction of 5'-HBGF-1 flanking region/CAT constructions is presently underway to search for hormone response elements within the HBGF-1 gene by transfection into DDT1 and LNCAP cells.

E 330 CHARACTERIZATION AND UTILIZATION OF A PANEL OF ISOFORM-SPECIFIC MONOCLONAL ANTIBODIES TO PDGF, Charles E. Hart, Mason Bailey, Dee Curtis, Mark J. Murray, Sherri Osborn, Daniel F. Bowen-Pope*, Elaine Raines*, Russell Ross*, Ronald A. Seifert*, and John W. Forstrom, ZymoGenetics, Inc., 4225 Roosevelt Way N.E., Seattle, WA 98105, *Department of Pathology, University of Washington, Seattle, WA 98195

We have developed a panel of monoclonal antibodies to Platelet-derived growth factor (PDGF). These antibodies have been characterized for cross-recognition to the three potential dimeric forms of PDGF (AA, AB, BB). By using antibodies which have selected specificity for one or more of the isoforms of PDGF we have been able to detect and immunoaffinity purify the individual dimeric forms of PDGF from human platelets. We have used the immunoaffinity purified forms of PDGF, along with recombinant-derived AA and BB, to monitor differences in receptor binding and biological activities for the three forms of PDGF. This data suggests that there are multiple cell-surface receptors for PDGF which are expressed at different ratios on different cell types. It is possible that the differences in receptor expression account for the variation in biological responses reported for the three forms of PDGF. We have also developed isoform-specific ELISA's to allow us to detect the presence of the three dimeric forms of PDGF in various biological fluids.

E 331 INACTIVATION OF GROWTH GENE EXPRESSION IN DICTYOSTELIUM
Hamdy Hassanain and Will Kopachik, Michigan State University, Department of Zoology, E. Lansing, MI. 48824

The cellular slime mold, Dictyostelium discoideum, is a useful model for studying the regulation of growth cell-specific gene expression because cells cease division while they differentiate. A cDNA library made to vegetative cell transcripts was used to identify transcripts preferentially expressed in the vegetative cells. The levels of growth cell-specific mRNA fall dramatically during aggregation early in development. However, the levels do not fall simply as a result of starvation or aggregation specific cell contact. Rather, cells must be deprived of amino acids and cAMP administered to 50nM periodically in pulses, to mimic cAMP signal-relay in aggregation. This effect can be blocked either with cAMP-S (a non-hydrolyzable cAMP analogue) or adenosine both of which prevent cAMP binding to the cell surface receptor. It is also blocked in aggregateless mutants known to be defective in a G protein. The inactivation of growth cell-specific genes can also occur inappropriately in vegetative cells if the cell cycle is arrested by drugs or thermosensitive growth mutations. Together these results suggest that the regulation of these genes is balanced by positive signals acting against negative signals transduced through the cAMP receptor.

E 332 TRANSFORMATION OF HUMAN MAMMARY EPITHELIAL CELLS BY POLYOMAVIRUS MIDDLE T ANTIGEN, Dominique Davidson, Mary–Anne Dobrovolny and John A. Hassell, Institute for MolecularBiology and Biotechnology. McMaster University, Hamilton, Ontario L8S 4K1 Canada

To study transformation of human cells, we have constructed double–replacement adenovirus vectors that carry an oncogene in the E1A/E1B region and a dominant–selectable marker in the E3 region of the adenoviral genome. We have used one family of such adenovirus vectors to nonselectively introduce the polyomavirus (PyV) oncogene products (large, middle and small T antigen) individually and collectively into an established human mammary cell line, 184A1N4, derived from normal mammary breast tissue by benzo[a] pyrene treatment. The human cell line grows in quasi–defined medium containing insulin, hydrocortisone, EGF and 0.5% fetal bovine serum. However, increase of the serum concentration to 10% and removal of the other growth factors leads to cell death, unless the cells are transformed by an oncoprotein such as the SV40 T antigens. We observed that cell lines which express PyV middle T antigen, or all three T antigens, divide in medium containing 10% FBS. Neither large nor small T antigen expressing cell lines display this phenotype. Therefore, middle T antigen alone is sufficient to transform these cells as defined by this assay. Furthermore, cells transformed by middle T antigen condition the medium containing 10% fetal bovine serum to allow the growth of the non–transformed parent cell line. We are currently investigating the molecular basis for the phenotype conferred by middle T antigen in these cells.

E 333 THE GENERATION OF LYMPHOKINE-ACTIVATED KILLER (LAK) CELLS AGAINST HUMAN PRIMARY BRAIN TUMORS. Roberta L. Hayes, Ph.D., William N. Harrington, M.D., Ellery Moore, & Maxim Koslow, M.D., New York University Medical Center, Depts. of Neurosurgery & Pathology, & the Division of Neuro-Oncology, NYU Kaplan Cancer Center, New York, NY 10016.
We have studied the ability of recombinant human cytokines, such as, Betaseron (rIFN-B, Triton Biosciences) to augment the production of lymphokine-activated killer (LAK) cell activity derived from the peripheral blood (PBL) of normal healthy donors. PBL were activated for 4 days in serum-free medium (Aim V, GIBCO) in the presence of optimal or sub-optimal amounts of rIL-2, plus variable doses of IFN-B. No synergy in the development of LAK effector function was observed using doses of IFN-B ranging from 5-2000 Units/ml, and sub-optimal doses of rIL-2 as low as 50 U/ml. In addition, we have analyzed independent clones of the glioblastoma U-373MG, and the medulloblastoma cell line TE671, as target cells for both LAK and natural killer (NK) cell mediated lysis (ie., without rIL-2 activation, or effector cell culture). Thus far, none of the glioblastomas that we have tested have been sensitive to NK cytolysis. Surprisingly, however, the adherent TE671 cell line, also derived from a solid brain tumor, is an excellent NK target. In addition, all donors demonstrated good LAK lysis of the TE671 clones, while there was considerable variability in the level of LAK sensitivity in the clones of the U373 glioblastoma between donors. Finally, in contrast to the lack of an effect upon the generation of lytic effector cells, the pretreatment of the tumor target cells with IFN-B (1000-2000 Units/ml) for 48-72 hours substantially increased their sensitivity to NK and/or IL-2 activated LAK cell-mediated lysis.

E 334 EFFECTS OF TRANSFORMING GROWTH FACTOR-β (TGF-β) ON GROWTH AND DIFFERENTIATION, AND PRESENCE OF SPECIFIC TGF-β RECEPTORS IN HUMAN SQUAMOUS CARCINOMA CELLS, Charles D. Hébert and Linda S. Birnbaum, National Institute of Environmental Health Sci., Res. Triangle Park, NC 27709 and University of North Carolina, Chapel Hill, NC 27559.
Transforming growth factor-β has been shown to inhibit the growth of all normal and many cancerous epithelial cell lines in culture. The mechanism by which this inhibition takes place, however, is unknown. In this study, four human squamous carcinoma cell lines were tested for their sensitivity to growth inhibition and induction of differentiation by TGF-β. DNA synthesis and monolayer growth in two of these cell lines, SCC-15G and SCC-25, were strongly inhibited by concentrations of TGF-β as low as 10 pM. Inhibition of DNA synthesis was apparent by 8 hours in SCC-15G and by 2 days in the slower-growing SCC-25 cells. Clonal growth was also inhibited in SCC-15G, but not in SCC-25 cells. The inhibition of monolayer growth in SCC-15G cells was reversible by removal of TGF-β-containing medium, but was irreversible in SCC-25 cells. DNA synthesis and monolayer growth of SCC-12F cells were not affected by 1000 pM TGF-β. Monolayer growth of SCC-9 cells in high-density culture was moderately inhibited by TGF-β, but no effect on growth of logarithmically growing cells was observed. TGF-β did not induce differentiation, measured as increases in keratinization and formation of cross-linked envelopes, in any of the 4 cell lines. Specific high affinity TGF-β receptors were found in all 4 cell lines. Thus, the response of human squamous carcinoma cells to the growth-modulatory effects of TGF-β varies greatly between cell lines, and the lack of a response in some cell lines is not due to decreases in number or affinity of specific receptors on the cell surface.

E 335 IGF-II MAY ACT AS AN AUTOCRINE GROWTH FACTOR IN A HUMAN RHABDOMYOSARCOMA CELL LINE. Lee J. Helman, Mark A. Israel, and Osama M. El-Badry, Molecular Genetics Section, Pediatric Branch, National Cancer Institute, Bethesda, Maryland 20892.
IGF-II has previously been shown to act as a mitogen and to inhibit the differentiation of normal human myoblasts. The mRNA encoding this growth factor is expressed in rhabdomyosarcoma, an embryonal tumor derived from myoblasts. We have therefore studied the role of IGF-II in the pathogenesis of this tumor. In 5/5 tumor specimens and 1/2 cell lines we have examined, we detected mRNAs encoding both IGF II and the type I IGF receptor, the receptor through which the mitogenic activity of IGF II is thought to be mediated. To examine the possibility that IGF II may act as an autocrine growth stimulator in some rhabdomyosarcoma tumors, we evaluated the growth of a human embryonal rhabdomyosarcoma cell line, RD, in chemically defined, mitogen-free tissue culture media. Since this cell line grew at a similar rate in media with and without serum, and we had demonstrated that the RD cell line expressed IGF II mRNA, we evaluated the ability of αIR3, a monoclonal antibody that recognizes the IGF-I receptor, to inhibit the growth of RD cells. In these experiments, we found a 20% decrease in cell growth in αIR3 treated cells by using a thymidine incorporation bioassay. IGF-II may act as an autocrine growth factor in the human embryonal rhabdomyosarcoma cell line RD and may contribute to the unregulated growth of this tumor.

E 336 IN VIVO LABELLING OF HUMAN GLIOMA XENOGRAFTS IN ATHYMIC MICE WITH 125-I-EPIDERMAL GROWTH FACTOR (EGF). Emile M. Hiesiger, M.D. and Roberta L. Hayes, Ph.D. New York University Medical Center, Depts. of Neurology and Neurosurgery, & Division of Neuro-Oncology, NYU Kaplan Cancer Center, New York, NY 10016.
Approximately, 30-50% of malignant gliomas express EGF receptors (EGF-R). These tumors have been successfully imaged in patients with single photon emission computed tomography (SPECT) scanning using 111-In-anti-EGF receptor monoclonal antibody. Moreover, this same antibody has been used to deliver 125-I as radiotherapy in patients with high grade astrocytomas. Because of a lower molecular weight and Kd, EGF would be expected to cross the partially disrupted blood brain barrier of malignant brain tumors and bind with greater affinity to cell surface EGF-R than would anti-EGF-R monoclonals. We have investigated the biodistribution and binding of 125-I-EGF in five athymic Swiss nu/nu mice implanted simultaneously intracerabrally and in flank with U373-MG, an EGF-R positive anaplastic astrocytoma. Fourteen days post tumor implantation, when the animals appeared moribund, they were anesthetized and injected with 125-I-EGF via the tail vein. One hour post injection, the animals were sacrificed, and the tumors and organs were removed and plasma was obtained for the analysis of radioactivity. Tissue homogenates were analyzed electrophoretically by SDS-PAGE, and Western blotting. Brain tumor uptake of radiolabelled EGF was 3.25 fold greater than control brain and 65% that measured in flank tumors. These data suggest that EGF given in vivo may localize to brain or systemic tumors which express EGF-R. If delivery of intact EGF to tumors is feasible, labelled EGF may be a useful ligand for imaging tumors and selectively delivering antitumor agents.

E 337 EFFECTS OF DIFFERENTIATION ON THE BINDING OF INSULIN-LIKE GROWTH FACTORS (IGFs) BY A HUMAN GERM CELL TUMOR (TERA-2). K S Hirsch[1], J F Fleck[2,3], C A Frolik[1], S V Benenati[2] ,and G W Sledge Jr[2],. [1] Lilly Research Laboratories, Eli Lilly and Company, Indianapolis IN 46285. [2]Department of Medicine, VA Medical Center and Indiana University, Indianapolis, IN 46202, [3] National Research Council of Brazil (CNPq). Undifferentiated Tera-2 cells undergo differentiation when exposed to 2.1 mM diflouromethylornithine. These cells exhibit an altered morphology, a decreased growth rate and an increased expression of several differentiation antigens. When incubated with [^{125}I]-IGFs the differentiated cells bound 2-3-fold more IGF-II than untreated cells and showed a significant decrease in IGF-I binding. The binding of IGF-II was 20-fold greater than IGF-I. [^{125}I]-IGF-II binding was blocked by the addition of unlabeled IGF-II but not by IGF-I while [^{125}I]-IGF-I binding was blocked by either IGF-I or IGF-II. In a competitive binding assay, IGF-I failed to compete for the IGF-II binding sites. Undifferentiated cells exhibited a single class of IGF-II binding site ($3.5X10^5$ sites/cell and a K_D of $6.9X10^{-9}M$). Differentiated cells expressed two classes of IGF-II binding sites (K_D = $5.7X10^{-10}M$, $2.5X10^5$ sites/cell and K_D = $2.3X10^{-8}M$, $1.4X10^7$ sites/cell). In chemical cross-linking experiments, [^{125}I]-IGF-I bound to a 130 KDa protein, the anticipated molecular weight of the α-subunit of the type I IGF receptor. The binding of [^{125}I]-IGF-I was blocked equally by IGF-I and IGF-II. Labeled IGF-II bound to the 250 KDa type II receptor and a number of lower molecular weight proteins. Only IGF-II blocked the binding of [^{125}I]-IGF-II. These data suggest that differentiation of Tera-2 cells is accompanied by changes in the binding of the insulin-like growth factors which may be important in the expression of the differentiated phenotype.

E 338 ANTI-TUMOR ACTIVITY OF AN ANTI-EGF RECEPTOR MONOCLONAL ANTIBODY AND ANTIBODY-*Vinca* IMMUNOCONJUGATE. David A. Johnson, Magda C. Gutowski, Stephen L. Briggs, Daniel V. Fix, and Aster B. Henry.
Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.
The monoclonal antibody M225 reacts with the epidermal growth factor receptor (EGFr) which is expressed at elevated levels on human squamous carcinomas (Sobol et al JNCI 79(3) 403, 1987). The antibody has been shown to block binding of EGF without stimulating tyrosine kinase activity, and to suppress tumor cell growth (Masui et al Cancer Res 46:5592, 1986). We wished to investigate the potential tumor suppressive activity of M225 in comparison to a corresponding drug immunoconjugate. The immunoconjugate was constructed by conjugating *Vinca* hydrazide to the oxidized carbohydrate moiety of affinity purified M225 antibody as detailed by Laguzza et al (J. Med. Chem. IN PRESS) resulting in a conjugate designated M225-DAVLBHYD. M225-DAVLBHYD retained antigen binding capacity which was similar to the unmodified antibody. *In vitro* cytotoxicity studies indicated that the drug moiety of the conjugate retained potency, the conjugate inhibiting 50% of tumor cell growth at a concentration of approximately 0.01 ug (vinca content)/ml. Unmodified antibody, however, had minimal tumor suppressive effects under the conditions of this assay. The M225-DAVLBHYD conjugate and unmodified antibody were then tested for their ability to regress human tumor nude mouse xenografts. The conjugate effectively regressed established tumors to zero at doses as low as 0.5 mg (vinca content)/kg. This was superior to the anti-tumor activity observed with free drug or mixtures of free drug and unmodified antibody. Equivalent protein doses of unmodified M225 antibody had minimal anti-tumor effects, though higher doses of unmodified antibody were growth suppressive.

E 339 HUMAN GALACTOSYLTRANSFERASE ACTIVATOR: EXTENSIVE SEQUENCE HOMOLOGY WITH HUMAN AND YEAST CELL DIVISION CONTROL PROTEIN KINASES, Vincent J. Kidd, Bruce Bunnell, Donald Adams and S. Kris Hollinger, Department of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294 Human β1-4 galactosyltransferase has been implicated in a number of processes including cell-cell interactions, fertilization, differentiation and cell proliferation, and neoplasia. We have previously shown that cell-surface galactosyltransferase is an integral part of β-agonist stimulated cell growth in a rat model system. One component of a galactosyltransferase holoenzyme complex, galactosyltransferase activator (GTA), has now been sequenced and expressed in eukaryotic cells. The predicted amino acid sequence of the 3.5 kb cDNA is 45-55% homologous to human 2Hs and yeast cdc 28 protein kinases. When expressed in eukaryotic cells, this cDNA stimulates the activity of galactosyltransferase 4-8 fold in a specific manner. It also leads to the re-localization of galactosyltransferase to the cell surface. This cDNA is part of a multi-gene family whose members are expressed at various times during development and normal cell growth. One of the GTA transcripts in normally expressed in immunoglobulin secreting B cells, but not at earlier time points. However, in specific B cell neoplasias ectopic expression (40-50 fold) of this mRNA accompanies apparent structural alterations in its corresponding gene. This ectopic expression correlates with higher levels of cell-surface galactosyltransferase and is related to the proliferative capacity of these tumor cells.

E 340 ROLE OF THE PROTO-ONCOGENE C-FOS IN THE INDUCTION OF THE DIFFERENTIATION OF WEHI-3B D^+ CELLS, Ivan C. King, Christina Gamba-Vitalo and Alan C. Sartorelli, Department of Pharmacology and Developmental Therapeutics Program, Comprehensive Cancer Center, Yale University School of Medicine, New Haven, CT 06510.

A rapid increase in c-fos expression has been reported to occur during monocytic differentiation of the murine monomyelocytic leukemia WEHI-3B D^+. To determine whether production of the c-fos gene product is sufficient for the induction of the differentiation of WEHI-3B D^+ cells, we introduced a DNA construct containing the human metallothionein promoter and the mouse c-fos gene into these cells. Integration of exogenous c-fos into the genome of these leukemic cells was demonstrated by Southern analysis. c-Fos RNA expression was increased from 2- to 8-fold after cells were treated with 60 uM cadmium chloride for 16 hr. In contrast, cadmium did not induce c-fos expression in parental cells. Treatment of WEHI-3B D^+ cells containing exogenous c-fos with cadmium for 3 days did not increase cellular differentiation, as determined by reduction of nitroblue tetrazolium and the expression of non-specific esterase. Furthermore, the transformants did not lose their ability to differentiate in response to the granulocytic inducers, retinoic acid and aclacinomycin A. The findings indicate that increased expression of c-fos in WEHI-3B D^+ cells is not sufficient to induce macrophagic differentiation nor necessary for granulocytic maturation.

E 341 A BIOLOGICALLY ACTIVE SYNTHETIC PEPTIDE CREATED FROM TWO NONCONTIGUOUS DOMAINS OF RETROVIRAL P15E, William Kloetzer, Don Wegemer, Karen Kabat, John Warner and Robert Naso. The Johnson & Johnson Biotechnology Center, San Diego, CA 92121.
Immunosuppression often accompanies persistent viremia of cats infected with feline leukemia virus (FeLV). Some of the clinical signs are directly attributed to the envelope protein p15E. A 17 aa immunosuppressive synthetic peptide (ISP) selected from a highly conserved region of retroviral p15E is biologically active when chemically cross-linked to BSA (Science 230:453 1985). We describe biological activities of a 29 aa carrier-free peptide (FISP) on con A stimulated murine splenocytes. FISP levels of 11μM to 22μM maximally suppress while peptide levels greater than 22μM have no effect or stimulate [^{3}H]TdR uptake by mitogen activated splenocytes. Passage of splenocytes through nylon wool effectively removes a FISP refractile population of con A responsive cells. Further T cell enrichment by monoclonal antibody mediated cytolysis shows that $CD4^+$ cells are the major splenocyte population suppressible by FISP. Consistent with this interpretation are results showing that FISP inhibits T_h cell dependent *in vitro* boosting of antibody production as determined in hemolytic plaque assays. Little or no suppression of [^{3}H]TdR uptake was detected in 1 and 2 way MLRs, IL-1B stimulated U373 cells or IL-2 stimulated CTLL-2 cells. The FISP sequence contains a His, Arg, Lys rich domain of p15E linked to the NH_2-terminus of FeLV ISP. Analysis of different hybrid peptides indicates that the polycationic tail probably contributes a nonspecific charge effect while the ISP contribution to biological activity is sequence specific. The availability of a synthetic, biologically active peptide should prove useful in defining the cellular mechanism by which viral p15E mediates its immunosuppressive effects.

E 342 EVIDENCE FOR A Gp PROTEIN IN ACTIVATED HUMAN B LYMPHOCYTES.
°Dominique Renard, *Elisabeth Petit-Koskas, *Elisabeth Génot, °Josiane Poggioli and *Jean-Pierre Kolb. °U274 Inserm, Physiologie et Pharmacologie Cellulaire, Orsay and *U196 Inserm, Recherche sur les Interférons, Institut Curie, Paris, France.

Hydrolysis of polyphosphoinosides, yielding the second messengers inositol-tris-phosphate and Ca^{++}, is involved at several steps during the process of human B cell activation. This metabolic way is stimulated by anti-μ antibodies which provide a first signal of activation and also, as we have shown previously, by the low-molecular weight B cell growth factor (LMW-BCGF or 12 kDa BCGF) which elicits the G1-S transition.

B cell blasts, recovered and purified after stimulation with anti-μ antibodies, were loaded with tritiated inositol and permeabilized with saponine. Increasing the concentration of free Ca^{++} above $3x10^{-6}$M resulted within 10 minutes in a marked increase in the amount of labelled inositolphosphates in those cells. This increase was also observed following the addition of 100 μM GTP-γS, a non-hydrolysable analogous of GTP. These results indicate that a GTP-binding protein of the Gp type can be non-specifically activated in human B cell blasts by these pharmacological reagents.

Various growth factors are now investigated in this system, as the effects of ADP-ribosylating molecules, such as the cholera and pertussis toxins.

E 343 ANTIPROLIFERATIVE ACTION OF HUMAN INTERFERON-GAMMA ON HUMAN EPIDERMOID CARCINOMA CELL LINE A431, Rakesh Kumar and John Mendelsohn, Laboratory of Receptor Biology, Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, NY 10021 The growth-inhibitory effect of human interferon-gamma (IFN-Y) was investigated in human epidermoid carcinoma line A431. During seven days treatment of A431 cells with IFN-Y (10-1000 units/ml), there was up to 90% inhibition of cell growth, in a dose and time dependent manner. The antiproliferative effect of IFN-Y is accompanied by profound morphological alterations from day 3 onward, and prolongation of the G_0/G_1 phase of cell-cycle. Immunofluorescence studies using an anti-human keratinocyte antibody (H-14) revealed appearance of the differentiated keratinocyte phenotype in the interferon treated cells. Exposure of A431 cells to IFN-Y resulted in elevation of expression of TGF alpha mRNA. We also examined changes in the activity of 2-5 oligo(A) synthetase, an interferon-induced enzyme, under these growth inhibitory conditions. Upon 18 hours of treatment with 10-1000 units/ml IFN-Y, 2-5(A) synthetase activity was increased by two-fold, followed by a further two-fold increase by day 3. Experiments are underway to explore the role of the INF-induced changes in TGF alpha expression upon the altered growth pattern and the observed variations in 2-5 oligo(A) synthetase activity.

E 344 EXPRESSION OF INDUCIBLE MEMBRANE-ANCHORED INSULIN RECEPTOR KINASE ENHANCES DEOXYGLUCOSE UPTAKE. David E. Lebwohl and Ora M. Rosen, Program in Molecular Biology and Dept. of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY. We have transfected mouse L cells with a recombinant membrane-anchored insulin receptor kinase(called MARK), under the transcriptional control of a glucocorticoid responsive element (MMTV promoter.) The transfected construct includes only 16 extracellular residues, with the transmembrane and intracellular kinase domains of the human insulin receptor(hIR) cDNA (aa -27 to 12, 965 to 1343, predicted MW 56kDa with signal sequence, 53kDa without.) Transfected cells which are exposed to dexamethasone express a doublet at ≈54kDa which (1) reacts with anti-peptide antisera raised to hIR sequences, (2) localizes to the particulate fraction, and (3) possesses ligand (insulin)-independent tyrosine kinase activity. When cell extracts were analyzed by immunoblotting with anti-phosphotyrosine antisera, two novel phosphotyrosine-containing proteins were detected in steroid-treated MARK transfectants: an ≈54kDa protein, the recombinant autophosphorylated protein, and an 185 kDa protein which corresponds in size to a known endogenous substrate for the insulin receptor. Control studies were performed with the nontransfected parent line, and with L cells transfected with an inactive kinase. Dexamethasone induced no change in the phosphotyrosine-containing proteins in the two control lines. Deoxyglucose uptake was studied in these cell lines. Dexamethasone induced a 1.8-2.2 fold increase in DOG uptake in the MARK cells, but not in the control lines. These studies demonstrate that a membrane-anchored insulin receptor kinase, devoid of virtually the entire extracellular domain of the insulin receptor, is sufficient to induce enhanced deoxyglucose uptake. Further studies of the physiologic functions of MARK, as well as a soluble kinase derived from it, are underway.

E 345 A NOVEL MECHANISM OF PATHOLOGICAL ACTIVATION OF THE INTERLEUKIN-3 GENE IN AN IN VIVO-DERIVED MURINE MYELOID LEUKEMIA, Kevin B. Leslie and John W. Schrader, The Biomedical Research Centre, University of British Columbia, Vancouver, BC, Canada, V6T 1W5. Previously we have described the independent rearrangement and activation of two lymphokine genes (interleukin-3 and granulocyte-macrophage colony-stimulating factor) in related but distinct clones from the in vivo-derived murine myelomonocytic leukemia, WEHI-274, resulting in autocrine growth behaviors mediated by those growth factors. Both rearrangements have occurred 5' to the respective growth factor gene giving rise to grossly abnormal transcripts of those genes. The IL-3 gene rearrangement has introduced an abnormal promotor 5' to the gene resulting in a transcriptional 'read-through' into the IL-3 gene and generation of an abnormal 8kb mRNA species instead of the normal 1.3kb, T cell-derived species. Data will be presented to describe a novel mechanism of pathological gene activation mediated by an endogenous retroviral-like sequence in this in vivo derived myeloid leukemia.

E 346 THE COMMON ACUTE LYMPHOBLASTIC LEUKEMIA ANTIGEN (CALLA) IS ENZYMATICALLY ACTIVE NEUTRAL ENDOPEPTIDASE 3.4.24.11, Michelle Letarte, Sonia Vera, Rosette Tran, Russell J. Onizuka, Elizabeth J. Quackenbush, Roderick R. McInnes, Stefan Carrel*, and C. Victor Jongeneel*, Hospital for Sick Children, Toronto, Canada, and *Ludwig Institute for Cancer Research, Lausanne, Switzerland.

CALLA (CD10) is a characteristic marker of non-T ALL, and is also abundant in normal kidney. We purified CALLA from human kidney and isolated a cDNA clone reactive with two oligonucleotide probes corresponding to two distinct peptides. The amino acid sequence translated from the CALLA cDNA is identical to that of human neutral endopeptidase (NEP, EC 3.4.24.11, enkephalinase) (Malfroy et al., FEBS Letters 229:206, 1988), and to that of CALLA isolated from leukemic cells (Shipp et al., PNAS 85:4819, 1988). NEP is a membrane-bound zinc metallopeptidase capable of hydrolyzing a number of bioactive peptides. By flow cytometry, a monoclonal antibody produced against rabbit NEP reacted selectively with leukemia and melanoma cell lines expressing CALLA on their surface. A glycoprotein of apparent M_r 100,000 could be immunoprecipitated from surface labeled NALM-1 leukemia or Mel-1477 melanoma cells with monoclonal antibodies to NEP or CALLA. mRNAs hybridizing to a NEP-specific probe were present in CALLA-positive leukemia and melanoma cell lines, but absent from CALLA-negative lines. NEP enzymatic activity was detected on intact cells from CALLA-positive lines, but not CALLA-negative lines. The activity was blocked by two selective inhibitors of NEP, thiorphan and phosphoramidon. CALLA antigen purified from the NALM-6 leukemic cell line retained NEP activity; this activity was completely blocked by selective inhibitors of NEP. Thus the CALLA antigen present at the surface of leukemia and melanoma cell lines is an enzymatically active neutral endopeptidase.

E 347 EXPRESSION OF PDGF A-AND B-CHAIN mRNA IN SOMATIC CELLHYBRIDS, Per Leveen, Christer Betsholz, Bengt Westermark, Department of Pathology, University of Uppsala, Sweden.

We have performed experiments with hybrid cell lines in order to study the regulation of PDGF A- and B-chain gene expression in human tumor cells. The hybrids were obtained as fusion products between the human melanoma cell line WM115 which expresses both PDGF A- and B-chain mRNA, and the HAT-sensitive, oubaine resistent hamster fibroblast cell line Wq3H which does not express A- or B-chain mRNA. Out of ten hybrid clones one (Cl 4) was found to contain both the human A- and B-chain genes as determined by Southern blot analysis. Northern blot analysis of nine subclones derived from Cl 4 showed that the human B-chain gene was completely suppressed in seven and weakly expressed in two subclones. In contrast, the human A-chain mRNA was present in all subclones at a similar level of that in WM115. Furthermore, treatment of different hybrid clones with the protein synthesis inhibiting agent cycloheximide induced the B-chain gene only in clones containing the human B-chain allele. The A-chain mRNA level was on the other hand not influenced by cycloheximide treatment. These results indicate that a negative trans-acting control element(s) derived from the hamster genome has suppressed the human B-chain gene as well as that the A-chain gene is regulated by another control mechanism since it is expressed in the hybrids and uninfluenced by cycloheximide treatment.

E 348 BIOLOGICAL PROPERTIES OF MUTATED HUMAN GM-CSF PROTEINS, A.F. Lopez, M.F. Shannon, L.B. To and M.A. Vadas, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia, 5000

Human granulocyte (G)-macrophage (M) colony-stimulating factor (CSF) is a glycoprotein of 19,000 central to hemopoiesis in that (i) it acts on different target cells such as granulcoytes (G), monocytes (M) and eosinophils (Eo) and (ii) it stimulates different functions of these cells, such as progenitor cell proliferation and differentiation, and mature cell effector mechanisms. Despite this pleiomorphism both in terms of target cells and function, little is known about the region/s of this molecule necessary for its activities. Using site-directed mutagenesis and a variety of biological and radio-receptor assays we have found that deletion of 11 amino acids (14-24) from the predicted first alpha helix of the mature GM-CSF molecule greatly impaired its ability to stimulate G, M and Eo colonies, and mature G and Eo function. In contrast, deletion of 21 amino acids (60-80) encompassing most of a predicted third alpha helix did not result in a decrease of any of these GM-CSF functions. Detailed analyses of the first alpha helix revealed that deletion and substitutions of two amino acids, Gln at position 20 and Glu at position 21 resulted in preferential impairment of some but not all the functions of GM-CSF. These results identify structural elements necessary for GM-CSF function raising the possibility of deleting relatively large portions of the GM-CSF molecule without affecting its function. In addition, impairment of some but not all of the functions of GM-CSF by single amino acid substitutions suggests heterogeneity in the interaction of GM-CSF with GM-CSF receptors on myeloid cells.

E 349 BIOLOGICALLY ACTIVE GLYCOSYLATED TGF-α RELEASED BY AN ESTROGEN RECEPTOR NEGATIVE HUMAN BREAST CANCER CELL LINE. Ruth Lupu, Robert B.Dickson and Marc E. Lippman. Lombardi Cancer Research Center, Georgetown University, 3800 Reservoir Rd, Washington DC, 20007.

The estrogen receptor negative human breast cancer line MDA-MB 231 produce a transforming growth factor type α (TGF-α) and releases it into the medium as a soluble protein of an apparent molecular weight of 30 kDa. Based on tunicamycin treatment which converts this 30 kDa TGF-α form into a 21kD immuno reactive TGF-α species,we hipotesized that is a glycosylated novel precursor of TGFα.To test this hipothesis we carried out immynoprecipitation of the TGF-α sinthesized by in vitro translation of mRNA from MDA-MB 231 CELLS.A 21 kD polypeptide can be also immunoprecipitated after in vitro translation of mRNA from MDA-MB 231 cells. Processing of the in vitro translated precursor with canine microsomal membranes converts the translation product into a 30 kDa glycoprotein.

The synthesized TGF-α-like precursor can be cleaved with elastase,releasing smaller TGF-α species (10 kDa) with the biological properties of the mature 6 kDa TGF-α. A similar cleavage of the expected precursor (18kDa) TGF-α yielded a 6 kDa polypeptide from the Feline sarcoma virus-transformed Fischer rat embryo cells (FeSrV).

The identification and the biological potency of the human glycosylated form of TGF-α has been shown by induction of NRK soft agar colony formation and EGF cell membrane receptor binding. These studies demonstrate that the human mammary breast cancer cell line MDA-MA 231 releases a unique glycosylated form of TGF-α capable of strong mitogenic action in vitro.

E 350 **TWO N-*myc* POLYPEPTIDES WITH DISTINCT AMINO-TERMINAL PRIMARY STRUCTURES ENCODED BY EXONS II AND III.**

Tomi P. Mäkelä, Kalle Saksela and Kari Alitalo, *University of Helsinki, 00290 Helsinki, Finland.*

N-myc and c-myc are structurally and functionally related proto-oncogenes with distinct oncogenic spectra. c-myc is activated by retroviral integrations, chromosomal translocations or gene amplification; the only known mechanism of N-myc activation is amplification. The c-myc protein is synthesized from two translational initiation sites in the first and second exons of the gene (Hann, S. R., M. W. King, D. L. Bentley, C. W. Anderson, and R. N. Eisenman. 1988.Cell 52: 185-195). Loss of the longer of the c-myc polypeptides may be important in the pathogenesis of Burkitts lymphomas. We find that the N-myc protein from human tumor cell lines appears as two closely spaced polypeptide bands (p58-60) in SDS-PAGE after treatment with alkaline phosphatase to remove phosphorylation. An SV40 early promoter-driven expression vector lacking N-myc exon I produced similar polypeptides when expressed transiently in COS-7 cells. A polypeptide doublet was also produced from N-myc constructs deleted of up to two thirds of the 3' coding sequences, suggesting that the difference between the two N-myc polypeptides localizes close to their N termini. These results prompted us to define the N termini of the N-myc polypeptides by radiosequencing of the two protein bands and by site-specific mutagenesis of the 5' end of the long open reading frame. Our results show that the two polypeptides are derived from two alternative in-phase AUG initiation codons located at the 5' end of exon II. Both polypeptides are phosphorylated and localized to the nucleus even when expressed separately. The translational initiation codons of N-myc are only 24 bases apart in exon II, suggesting a possible explanation for the relative resistance of the N-myc gene to insertional mutagenesis and chromosomal translocations, which commonly activate the c-myc gene and alter its protein products whose corresponding translational initiation codons are located in separate exons 1.7 kbp apart.

E 351 HER-2/neu AMPLIFICATION AND/OR OVEREXPRESSION BY DNA, RNA AND PROTEIN ANALYSIS IN BREAST CANCER. G. Manenti, M.A. Pierotti, S. Andreola, F.M. Rilke, M.G. Da Dalt, S. Menard, M.I Colnaghi, D. Coradini, G. Di Fronzo, V. Quagliolo, *D.J. Slamon and G. Della Porta. Istituto Nazionale Tumori, Milano, *UCLA School of Medicine, Los Angeles.

The amplification and the expression of HER-2/neu oncogene on frozen tissue from 103 human breast tumors was analyzed with Southern blotting for DNA amplification, Northern blotting for RNA expression, and Western blotting for protein expression using a rabbit polyclonal antipeptide serum. With the same serum an immunohistochemical study was performed on paraffin embedded cancer tissue from the same patients. The results indicated that almost 30% of the samples presented amplification and/or overexpression of HER-2/neu. The amplilfied samples were overexpressed both at RNA and protein levels. Moreover, a significant correlation in detecting HER-2/neu overexpression was found between the molecular techniques and the immunohistochemical approach on paraffin embedded tissue. These results prompted us to analyze with the anti-HER-2/neu serum a group of 98 untreated, node-negative breast cancer patients with a significant time of follow-up and of which only paraffin embedded tissue was available. The results of the analysis showed a significant correlation between poor prognosis and immunostaining.

E 352 GENOMIC CHARACTERIZATION OF A MYELOID RELATED SEQUENCES, Wendy M. Mars and Grady F. Saunders, Department of Biochemistry and Molecular Biology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

Leukemia, like other cancers, is caused by an imbalance in the normal regulation of differentiation and division. In lymphoid malignancies, deregulation is known to occur by the abnormal juxtaposition of a differentiation-specific sequence with one that is normally regulated in the cell-cycle. Although examples of this mechanism are currently unknown in the myeloid leukemias, the discrepancy may simply reflect the fact that very few myeloid-specific genes have been thoroughly characterized. We previously isolated a myeloid-related sequence (*mrs*) from chronic myelogenous leukemia that is primarily expressed in promyelocytes, myelocytes, eosinophils and basophils. Analysis of the cDNA suggests the *mrs* gene encodes a 93 amino acid peptide. Thirty amino acids at the carboxy terminus correspond to HP-1, a protein with both toxic and growth-promoting activities that is normally found in human neutrophils and present in some lung tumors. We have now isolated and characterized genomic clones encompassing the normal *mrs* gene. Three genomic clones with *mrs* homology have been identified that each contain 15-16.5 kb of DNA. The majority of the structural gene is located within a 4 kb region of DNA; however, there is one intron spanning 10-11 kb in length located at the 5' end of the gene. The primary structures of the clones are currently being analyzed in detail and will be presented.

E 353 A DERIVATIVE OF STAUROSPORINE (CGP 41 251) SHOWS SELECTIVITY FOR PKC-INHIBITION AND *IN VITRO* ANTIPROLIFERATIVE AS WELL AS *IN VIVO* ANTITUMOR ACTIVITY, Thomas Meyer§, Urs Regenass§, Doriano Fabbro¶, Enrica Alteri§, Johannes Roesel§, Marcel Mueller§, Giorgio Caravatti§ and Alex Matter§, §Pharmaceutical Research Department, Pharma Division, CIBA-GEIGY Ltd., CH-4002 Basel and ¶Department of Research and Department of Gynecology and Obstetrics, University Clinic Medical School, CH-4031 Basel, Switzerland.

Analogs of staurosporine were synthesized and their ability to inhibit protein kinases examined. Staurosporine was found to be a potent but nonselective inhibitor of *in vitro* protein kinase C (PKC) activity (IC50 6.0nM). The derivative CGP 41 251 had reduced PKC activity with an IC50 of 50nM but showed a high degree of selectivity when assayed for inhibition of cyclic AMP dependent protein kinase (IC50 2.4nM), S6 kinase (IC50 5.0 μM) and tyrosine kinase specific activity of epidermal growth factor receptor (IC50 3.0 μM). Staurosporine and CGP 41 251 exerted growth inhibition in the human bladder carcinoma line T-24, human promyelocytic leukemia line HL-60 and bovine corneal endothelial cells at concentrations which correlated with *in vitro* PKC inhibition. In addition, both compounds inhibited the release at nontoxic concentrations of H_2O_2 from human monocytes pretreated with TPA. Tumor growth inhibition tests revealed significant antitumor activity ($2P<0.001$) at 1/10 of the maximal tolerated dose for both compounds. By contrast, a closely related derivative of staurosporine (CGP 42 700) was found to be inactive at concentrations >100 μM in all *in vitro* enzyme and antiproliferative assays as well as in animal tumor models. These data suggest an association between PKC inhibition and antiproliferative and antitumor activity.

E 354 THE ROLE OF THE RETINOBLASTOMA ANTI-ONCOGENE AND SELECTED GROWTH FACTORS IN GROWTH OF NORMAL AND NEOPLASTIC BONE CELLS, Beverly W. Miller, Matthew S. Blough, James H. McMaster, and Josef F. Novak, Orthopaedic Research Laboratory, *Allegheny-Singer Research Institute, Pittsburgh, PA 15212*

The loss of function of both alleles of the retinoblastoma susceptibility gene has been associated with the development of many osteosarcomas, as well as retinoblastoma. The expression of the Rb gene appears to be intimately associated with both the normal growth controls and oncogenic mechanisms of bone cells. The objective of our research is to establish a link between the expression of the Rb anti-oncogene and expression of growth factors which may be the targets of the Rb gene product in controlling growth.

Data from this laboratory indicate carbohydrate intolerance in osteosarcoma patients with normal insulin levels. Also, some osteosarcomas have been show to produce an insulin-like growth factor (IGF; Blatt et al., 1984). Therefore, preliminary experiments were focused on IGF-I and IGF-II. Southern hybridization analysis of these genes revealed restriction fragment length polymorphisms in two human osteosarcoma cell lines, G-292 and U2-OS. Preliminary Northern analysis of total RNA from these cells shows expression of the Rb message but not of IGF-II. Other human OS cell lines are being examined, as well as other growth factors, including PDGF, EGF, and TGF-beta. A murine model system, including normal primary osteoblasts, a normal osteoblastic cell line, and cloned osteosarcoma cells passaged as tumors and *in vitro*, is also being studied.

E 355 EGF RECEPTORS ARE PRESENT ON HUMAN LUNG CARCINOID CELL LINES, T. Moody, M. Lee, R. Kris, F. Bellot, J. Schlessinger, H. Oie and A. Gazdar. Dept. Biochemistry, George Washington Univ. Med. Sch., Washington, DC 20037, Rorer Biotechnology Inst., King of Prussia, PA 19406 and NCI-Navy Medical Oncology Branch, Bethesda, MD 20814. Previously, we found that some of the lung carcinoid cell lines were enriched in their neuroendocrine porperties and had high levels of dopa decarboxylase activity and bombesin-like peptides (Gazdar et al., Cancer Res. 48:4078 (1988). Here, we investigated if lung cancer cell lines have EGF receptors. ^{125}I-EGF did not bind to 3 small cell lung cancer (SCLC) cell lines examined but did bind to 4 non-small cell lung cancer (NSCLC) cell lines. In 2 squamous cell carcinoma, 1 large cell carcinoma and 1 adenocarcinoma cell lines, the EGF receptor density ranged from 50,000-150,000/cell. ^{125}I-EGF bound to a high and moderate density of sites using carcinoid lines NCI-H727 and H720 but not cell lines H835 or H679. Specific ^{125}I-EGF binding to cell line NCI-H727 was inhibited half maximally by 20 ng/ml unlabeled EGF and Scatchard analysis indicated that the density of binding sites was 120,000/cell. Specific ^{125}I-EGF binding was inhibited half maximally using 1 μg/ml of 108, a monoclonal antibody against the EGF receptor. The EGF receptors function as protein tyrosine kinases in that using SDS PAGE techniques, 100 ng/ml of EGF stimulated phosphorylation of major 170 Kdalton band. The ability of 108 to alter the growth of NSCLC cells will be discussed. These data indicate that NSCLC cell lines and some lung carcinoids, but not SCLC cell lines, have EGF receptors. Supported by NCI grants CA-42306 and CA-33767.

Differentiation; Growth Factors and their Receptors-II

E 400 THE CALCIUM SIGNAL FOR BALB/MK KERATINOCYTE TERMINAL DIFFERENTIATION INDUCES SUSTAINED ALTERATIONS IN PHOSPHOINOSITIDE METABOLISM WITHOUT DETECTABLE PROTEIN KINASE C ACTIVATION. Jorge Moscat, Timothy P. Fleming, Christopher J. Molloy, Stuart A. Aaronson, LCMB, NCI, NIH, Bldg. 37, Rm. 1E24, Bethesda, Maryland 20892

BALB/MK keratinocytes require EGF for proliferation and terminally differentiate in response to elevated extracellular Ca^{2+} concentrations. We show that a sustained activation of phosphoinositide metabolism is produced upon addition of Ca^{2+} to BALB/MK cultures. The pattern of inositol trisphosphate ($InsP_3$) isomers released in response to Ca^{2+} challenge appeared to be atypical. Ins(1,3,4)P_3 release was observed by 30 s and preceded any alteration in Ins(1,4,5)P_3 levels. Concomitant with the liberation of InsPs, an increased production of diacylglycerol (DAG) was observed. However, despite a three-fold increase in DAG levels detected even at 12 h after Ca^{2+} addition, no evidence of functional activation or down-regulation of protein kinase C (PKC) was found. This was established by measuring p80 phosphorylation, EGF binding and PKC levels by immunoblotting. Analysis of the Ca^{2+}-elicited DAG revealed that a significant proportion of the lipid consisted of an alkyl-linked glyceride molecular species. These results suggest that this DAG molecular species may play a role in the Ca^{2+}-induced differentiation program of BALB/MK cells through mechanisms independent of PKC.

E 401 TGF-β INHIBITION OF GROWTH FACTOR-STIMULATED MITOGENESIS AND C-MYC EXPRESSION IN HUMAN COLON CARCINOMA CELLS UNDER SERUM-FREE CONDITIONS, Kathleen M. Mulder, Mark J. Lynch, Karla E. Childress-Fields, Xochil H. Hinshaw, Mary K. Ramey, and Michael G. Brattain, Department of Pharmacology, Baylor College of Medicine, Houston, TX 77030

Previous work indicated that the growth control of poorly-differentiated human colon carcinoma cells by nutrients and polypeptide factors was altered relative to that of the well-differentiated cells under completely serum-free conditions. ^{3}H-Thymidine incorporation into TCA precipitable material and autoradiographic analysis indicated that nutrient replenishment alone was sufficient to initiate DNA synthesis in quiescent poorly-differentiated cells, whereas defined polypeptide growth factors produced no additional effect. In contrast, well-differentiated cells were mitogenically stimulated to a much greater extent by growth factors (epidermal growth factor + insulin + transferrin), than by nutrient replenishment alone. Peak mitogenesis occurred at 18-20 hours in both classes of cells. We now show that expression of the c-myc proto-oncogene was increased approximately 5-fold following growth factor addition to the well-differentiated cells. Maximal expression of c-myc occurred at 4 hours post-stimulation. In contrast, nutrients resulted in only a slight up-regulation of c-myc (1.8-fold) at approximately 90 minutes following addition. TGF-β abrogated the mitogenic responses to both growth factors (IC_{50} = 0.8 ng/ml) and to nutrients (IC_{50} = 0.2 ng/ml) in the well-differentiated cells. Moreover, TGF-β (10 ng/ml) completely abrogated the growth factor-stimulated up-regulation of c-myc. TGF-β may exert its proliferation inhibitory effects in responsive human colon carcinoma cells by altering cellular responsiveness to nutrients and polypeptide growth factors and/or by reducing the expression of competence genes such as c-myc.

E 402 ALTERATIONS IN SECOND MESSENGER PATHWAYS FOLLOWING INDUCTION OF THE RAS ONCOGENE, Suzanne K. Murphy, Claudia A. Iannotti, Amy K. Reiner, Arthur H. Lockwood*, Department of Biological Sciences, Philadelphia College of Pharmacy and Science, Philadelphia, PA 19104, *Long Island Jewish Hospital, New Hyde Park, NY. 11042

The second messenger systems involving cyclic AMP (cAMP) and phosphoinositide (PI) turnover are known to be fundamental for regulation of cell growth. We and others have demonstrated that expression of cellular oncogenes can perturb the function of these signal transduction pathways. In particular we have shown that one such transforming oncogene, H-ras, decreases cAMP levels and stimulates PI turnover in NIH3T3 cells. To study the early events after H-ras expression we have employed NIH3T3 cell lines in which H-ras expression is under the control of a steroid inducible promotor.

Following exposure to dexamethasone and induction of ras gene expression, alterations in cell morphology toward a malignant phenotype are observed. These cells have a more rapid growth rate, grow to a higher saturation density and do not exhibit the characteristic contact inhibition seen in untreated cells. Elevation of cAMP restores many aspects of growth and morphology to the ras expressing cells.

Analysis of phosphoinositide levels demonstrates a decrease in cellular levels of PIP and PIP_2 in cells induced to express the ras oncoprotein. Exposure of dexamethasone induced ras transformed cells to cAMP alters the levels of the phosphoinositides. We have previously demonstrated similar effects in 3T3 cells permanently transformed by H-ras. These results suggest that alterations in second messenger signalling systems are among the early events in the expression of the ras oncogene.

E 403 STIMULATION OF MOUSE UTERINE AND VAGINAL GROWTH AFTER IN VIVO EXPOSURE TO EPIDERMAL GROWTH FACTOR, Karen Nelson, Tsuneo Takahashi, LeMarquis Goods and John A. McLachlan, National Institute of Environmental Health Sciences, Laboratory of Reproductive and Developmental Toxicology, Research Triangle Park, NC 27709

Studies from our laboratory have provided evidence that epidermal growth factor (EGF) may have a role in the regulation of estrogen-induced mouse uterine growth (Endocrin. 122:2355 and 118:101) The studies described here were initiated to determine whether in vivo exposure to EGF would induce proliferation of mouse uterine and vaginal cells. Cholesterol based pellets containing EGF (2.5ng - 7.5µg) were implanted under the kidney capsule of ovariectomized female mice. EGF induced a dose-dependent stimulation of uterine and vaginal epithelial cell proliferation within 24 hr, as measured by [^{3}H]-TdR autoradiography. EGF also significantly enhanced uterine muscle DNA synthesis as well as produced a slight increase in stromal cell growth. The growth stimulatory effect of EGF appeared to be specific for uterus and vagina because a limited screening of other tissues did not reveal a growth response. Stimulation of uterine and vaginal DNA synthesis by EGF was found using ovariectomized, adrenalectomized and hypophysectomized mice which indicated that neither pituitary nor adrenal hormones were necessary for EGF-induced growth. A role of EGF in estrogen stimulation of uterine growth was also suggested by experiments where antibodies against EGF significantly inhibited estrogen-induced proliferation. Our data strongly suggests that EGF is a major component of an autocrine mechanism regulating estrogen-induced mouse uterine and vaginal growth.

E 404 SWITCH RECOMBINATION PRODUCTS OF SMALL POLYDISPERSE CIRCULAR (SPC) DNAs FROM MOUSE LYMPHOCYTES, Thai D. Nguyen, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, San Francisco, CA 94143

A population of small polydisperse circular (SPC) DNAs with various sizes from 5Kb to over 100 Kb were isolated and purified by ATP dependent DNase I treatment of Hirt extracts from mouse transformed lymphocytes. Oligomer nucleotide primers of two immunoglobulin switch regions, namely Sµ and Sδ2b, were used for polymerase chain reaction (PCR) using the SPC DNAs as templates. The amplified products were identified by M13 cloning. One clone shows six direct repeats in 140 bp with about 70% homology Sδ2a of immunoglobulin gene; the 3' sequence of the clone, 80 bp, can form inverted repeat stem structure to the direct repeats. A possible switch function of the clone and the recombination events in lymphocytes is discussed.

E 405 MECHANSIMS OF ACTIVATION OF PHOSPHOINOSITIDE HYDROLYSIS BY PLATELET-DERIVED GROWTH FACTOR IN BALB/c-3T3 CELLS. N.E. Olashaw, M.I. Wahl, G. Carpenter and W.J. Pledger. Dept. of Cell Biology and Biochemsitry, Vanderbilt University, Nashville, TN 37232. Platelet-derived growth factor (PDGF) stimulates the phospholipase C (PLC)-mediated cleavage of phosphatidylinositol-4,5 bisphosphate and the consequent formation of inositol phosphates in density-arrested BALB/c-3T3 cells. This action of PDGF is potentiated by pretreatment of cells with agents that elevate cellular cyclic AMP content. Recent studies assaying phosphoinositide-specific PLC activity in cell extracts immunoadsorbed to an anti-phosphotyrosine matrix demonstrated increased activity in material prepared from PDGF-treated as compared to control cultures. Immunoisolated PLC activity from PDGF-treated cells correlated in a dose- and time-dependent manner with PDGF-stimulated inositol trisphosphate (IP_3) formation in intact cells. Pretreatment of cells with the cAMP elevating agents forskolin and IBMX for 4 hr prior to addition of PDGF, however, enhanced PDGF-induced IP_3 production but did not increase immunoisolated PLC activity above that obtained with PDGF alone. Similar assays showed that pre-exposure of cells to forskolin and IBMX potentiated IP_3 formation in response to aluminum fluoride, a direct activator of G proteins that did not increase in vitro PLC activity. Inclusion of cycloheximide during the pretreatment period abrogated the effect of cAMP on both PDGF- and aluminum fluoride-stimulated IP_3 production. These data suggest that PDGF may increase IP_3 by inducing the phosphorylation of PLC at tyrosine and that cAMP augments PDGF-stimulated IP_3 production by a post-receptor mechanism unrelated to PLC phosphorylation and involving protein synthesis and perhaps G proteins.

E 406 IDENTIFICATION OF A HEPARIN-BINDING GROWTH FACTOR RECEPTOR IN DROSOPHILA MELANOGASTER, Bradley B. Olwin*, John S. Doctor+, and F. Michael Hoffmann+, *Department of Biochemistry and +McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706. Heparin-binding growth factors may play crucial roles in early embryonic development. To better understand the functions of HBGFs in embryonic development, we have begun characterization of HBGF homologs and HBGF receptors in Drosophila. A receptor for HBGF 2 has been identified and partially characterized in Drosophila S2 and KC cells. The 140 kDa receptor polypeptide was initially identified by chemical crosslinking of human recombinant ^{125}I-HBGF 2 to intact cells. Preliminary experiments suggest Drosophila HBGF receptor is present at low levels (< 5000 per cell) and binds mammalian HBGF 2 with high affinity (10-50 pM). In contrast to vertebrate HBGF receptors, Drosophila S2 and KC cells exhibit no detectable specific binding to bovine ^{125}I-HBGF 1. Analysis of Drosophila embryonic extracts has identified a Drosophila HBGF-like homolog that competes for ^{125}I-HBGF 2 binding to S2 cells, binds heparin and co-migrates with mammalian HBGFs on SDS-polyacrylamide gels.

E 407 EXPRESSION OF PDGF ISOFORMS IN CHO CELLS REVEALS DIFFERENCES IN PROCESSING, A. Östman[1], L.B. Rall[2], A. Hammacher[1], C. Betsholtz[3], B. Westermark[3], P. Valenzuela[2], C.-H. Heldin[1]. [1]Ludwig Institute for Cancer Research, Box 595, 751 23 Uppsala, Sweden. [2]Chiron Corp., 4560 Horton Street, Emeryville, Ca. 94608, USA, [3]University Hospital, 751 85 Uppsala, Sweden.

Three different isoforms of PDGF have been purified from natural sources, PDGF-AA, -AB and -BB. It has recently become clear that the different isoforms interact with different affinities with two distinct PDGF receptors and that the receptors transduce different signals. To study the biosynthesis of the different PDGF isoforms and to establish a system for the production of recombinant PDGF, CHO cells were transfected with PDGF cDNAs.

CHO cells were transfected with an expression vector containing the human PDGF A- and B-chain precursors. A cell line was established that produced all three dimeric forms of PDGF. PDGF-AA and -AB were processed to give final secreted forms of 30 kDa. In contrast PDGF-BB existed as two final products; a secreted form of 30 kDa and a cell associated form of 24 kDa.

To study the properties of the two forms of PDGF-BB a CHO cell line that stably expresses the human PDGF B-chain precursor was established. The secreted form of 30 kDa was purified and found to be processed as the B-chain of PDGF-AB purified from human platelets. The cell associated form was recovered in a membrane fraction and could be extracted from membranes with 1 M acetic acid. Both the secreted and the membrane associated forms were mitogenic and bound to both PDGF A- and B-type receptors.

A three-step procedure was developed for the purification of recombinant PDGF-AB from medium conditioned by the cell line expressing PDGF A- and B-chains. The purified recombinant PDGF-AB was structurally and functionally indistinguishable from PDGF-AB purified from human platelets.

E 408 BLASTEMAL CELLS ARE THE MAIN SOURCE OF IGF-II mRNA IN FETAL KIDNEY AND WILMS' TUMOR, Soonmyoung Paik, Neal Rosen, Marc E. Lippman, James F. Perdue*, and Douglas Yee, Medicine Branch, National Cancer Institute, Bethesda, MD 20892,*Holland Laboratory at the American Red Cross, Rockville,MD

Insulin-like growth factor II is thought to be the primary fetal somatomedin. We have studied changes in IGF-II mRNA expression pattern during human nephrogenesis using *in-situ* hybridization. We found that IGF-II mRNA is expressed in nephrogenic and stromagenic blastemal cells as well as in differentiated stromal cells and that epithelial differentiation is coupled with loss or decrease of IGF-II mRNA expression.

Because Wilms' tumor is believed to arise from nephrogenic blastemal cells, we have anlyzed five Wilms' tumor samples to study possible changes in IGF-II mRNA expression pattern. In the triphasic type, blastematous tumor cells and stromal cells showed IGF-II mRNA hybridization signals, whereas epithelial cells did not show signal. Thus IGF-II mRNA expression pattern closely mimics the pattern seen during normal nephrogenesis. But in monomorphous tubular type, epithelial tumor cells showed IGF-II mRNA expression. Biological and prognostic importance of this abnormal pattern of IGF-II mRNA expression needs to be studied further. In blastemal predominant type, all tumor cells expressed IGF-II.

Our data shows that blastemal cells are the main source of IGF-II mRNA from fetal kidneys and Wilms' tumors. This is in contrast to the previous report by Han et al (Science,236:193), which proposed a paracrine role for IGF-II in fetal development based on the cellular localization of IGF-II mRNA to stromal cells in the fetal tissues including kidney.

E 409 CONSTITUTIVE PRODUCTION OF MULTIPLE HEMATOPOEITIC GROWTH FACTOR ACTIVITIES FROM ADHERENT CELLS ISOLATED FROM MURINE FETAL LIVER, E.W. Palaszynski, Dept. of Biochemistry, George Washington University Medical Center, Washington, DC 20037

Adherent fetal liver cells from terminal pregnant NIH/Swiss mice were established in long-term culture to investigate their ability to produce factors which would promote the growth and proliferation of hematpoietic cell lines. These adherent cultures were termed H-8 Adherent. Morphologically these adherent cells are fibroblast-like in nature, are contact inhibited, grow in a cord-like fashion and can be easily trypsinized and transferred.

The semi-purified supernatants from these cultures contain several factor activities which supports the growth and proliferation of several IL-3 dependent cell lines, namely FW-311, 32D-cl-23 and NFS-60. The supernatants have been semi-purified by ammonium sulfate precipitation, ion exchange and molecular weight chromatographic proccedures. The molecular weight range of these semi-purified activities are 70 to 32 kilodaltons and are very stable upon storage. When these semi-purified activities are tested on the cell lines by ^{3}H-thymidine incorporation and specific antibody inhibition for known factors,it is found that they are not IL-1, IL-2, IL-3, GM-CSF or G-CSF. But, may be M-CSF, IL-4, IL-6, IL-7 or an unknown factor activity.

We are currently purifying and testing these activities further to determine their biochemical nature as well as their range of biological activities in bone marrow cultures and investigate their possible role in normal hematopoeitic growth and differentiation.

E 410 **EXPRESSION OF HAPTOGLOBIN-RELATED PROTEIN (*Hpr*) EPITOPES IN HUMAN BREAST CARCINOMA CORRELATES WITH INCREASED PHENOTYPIC MALIGNANCY.** Gary R. Pasternack and Francis P. Kuhajda, Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Previous studies showed that recurrence of human cancer correlated with immunohistochemical reactivity using a poorly characterized antiserum against a pregnancy plasma protein. Purification of the immunoreactive substance from pregnancy plasma and further characterization using peptide mapping, immunologic analysis, and protein microsequencing showed it to be the hitherto undescribed product of the haptoglobin related protein (***Hpr***) gene. The ***Hpr*** gene is located 2.2 Kb downstream from the conventional haptoglobin locus, contains a retrovirus-like element, and is duplicated in some individuals. Immunohistochemical staining with an affinity-purified polyclonal antibody raised against a synthetic peptide derived from the ***Hpr*** predicted α-chain sequence was performed on the previously studied collection of Stage I and Stage II breast cancer cases. Immunohistochemical reactivity correlated strongly with early recurrence ($p<.001$, Mantel-Cox and Savage tests on life table analysis). Moreover, staining of breast cancer was abolished by preincubation with ***Hpr*** purified from pregancy plasma, but not by preincubation with either haptoglobin I or II. Interestingly, expression of ***Hpr*** epitopes was generally limited to invasive tumor in those instances where there was co-existing carcinoma *in situ*, suggesting that the molecule bearing ***Hpr*** epitopes may be a biologically active mediator of the malignant phenotype, rather than simply a marker. (Supported by USPHS grants RO1 GM 36697, RO1 GM 46143, and a grant from the Andrew W. Mellon Foundation).

E 411 NUCLEAR RECEPTORS FOR THYROID HORMONE AND RETINOIC ACID AS SIGNAL MEDIATORS OF DIFFERENTIATION AND ONCOGENESIS, Magnus Pfahl, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

We have recently described the cloning and characterization of nuclear receptors for thyroid hormone and retinoic acid, two ligands which are important in development and differentiation. The two receptors belong to a multigene family which also includes the steroid hormone and vitamin D_3 receptors. These receptors are intracellular regulatory proteins which when complexed with their specific ligands recognize specific DNA elements and modulate transcription from linked promoters. The human thyroid hormone receptor (hTRα) cloned by us represents the cellular homologue to the viral ErbA protein. This receptor is expressed in various tissues and our recent data suggests that it is important in postnatal brain development. The human retinoic acid receptor (hRARε) recently described by us shows a considerable specificity for proliferating epithelial type tissues. Where erroneously expressed in the liver RARε may contribute to hepatocellular carcinoma development. Although RARε shows only limited homology with TRα it is able to activate transcription from thyroid hormone responsive elements. We have now started to analyze RARε and TRα expression in human tumor tissues.

E 412 SIGNAL TRANSDUCTION THROUGH THE EGF RECEPTOR TRANSFECTED IN IL-3-DEPENDENT HEMATOPOIETIC CELLS. Jacalyn H. Pierce, Marco Ruggiero, Timothy P. Fleming, Pier Paolo Di Fiore, Joel S. Greenberger, Lyuba Varticovski, Joseph Schlessinger, Giovanni Rovera, Stuart A. Aaronson, LCMB, NCI, NIH, Bldg. 37, Rm. 1E24, Bethesda, Maryland 20892

An expression vector for the epidermal growth factor (EGF) receptor was introduced into the 32D myeloid cell line, which is devoid of EGF receptors and absolutely dependent on interleukin-3 (IL-3) for its proliferation and survival. Expression of the EGF receptor conferred the ability to utilize EGF for transduction of a mitogenic signal. When the transfected cells were propagated in EGF, they exhibited a more mature myeloid phenotype than was observed under conditions of IL-3-directed growth. Moreover, exposure to EGF led to a rapid stimulation of phosphoinositide metabolism, while IL-3 had no detectable effect on phosphoinositide turnover either in control or EGF receptor-transfected 32D cells. Although the transfected cells exhibited high levels of functional EGF receptors, they remained nontumorigenic. In contrast, transfection of v-*erb*B, an amino-terminal truncated form of the EGF receptor with constitutive tyrosine kinase activity, not only abrogated the IL-3 growth factor requirement of 32D cells, but caused them to become tumorigenic in nude mice. These results show that a naive hematopoietic cell expresses all of the intracellular components of the EGF-signaling pathway necessary to evoke a mitogenic response and sustain continuous proliferation.

E 413 ACTIVATION OF C-RAF PROTEIN KINASE, A REGULATOR OF PEA 1 DEPENDENT TRANSCRIPTION, Ulf R. Rapp, Gisela Heidecker, W.B. Anderson[+], Bohdan Wasylyk , National Cancer Institute, Laboratory of Viral Carcinogenesis, Frederick, MD 21701; [+]Cancer Biology Branch, National Cancer Institute, Bethesda, MD 20892; Laboratory of Molecular Genetics, Institute of Chemical Biology, Strasbourg, Cedex, France. c-raf protein kinase can be activated reversibly by a multitude of growth factors or intracellular mitogens and irreversibly by structural changes of the gene including N-terminal deletion and site specific mutation within conserved region 2(CR2). Mutational activation of c-raf kinase also leads to its oncogenic activation. Reversible raf kinase activation is accompanied by translocation of raf protein from the cytosol to membranous fractions and the nucleus.

Expression of activated raf stimulates the activity of PEA1, a member of the c-jun gene family. Both v-raf and activated forms of either c-raf-1 or A-raf stimulate PEA1, suggesting that phosphorylation is involved in this activation. This good correlation between the abilities of various derivatives of raf to activate PEA1 and to transform cells strongly suggests that activation of PEA1 is an important event in transformation by raf.

E 414 A PHYSIOLOGICAL CELL CYCLE ARREST OCCURS IN THE G1, AND G2 PHASES AT THE ONSET OF SENESCENCE IN RODENT EMBRYONIC FIBROBLASTS, Timothy E.W. Riley, Sue C. Barnet, and Parmjit S. Jat, Ludwig Institute for Cancer Research, Courtauld Building, 91 Riding House Street, London, W1P 8BT, United Kingdom

Embryonic Fibroblasts display a finite *in vitro* life-span culminating in senescence.The progressive change from cycling to non-cycling cells occurs late in the approximate 29 divisions, that constitute the *in vitro* life-span of rodent embryonic fibroblasts. In Rat Embryonic Fibroblasts (REFs), immortalized with the tsA58 temperature sensitive large T antigen of SV40, senescence can be induced at the non-permissive temperature. Characteristically such cells arrest at both the G_1, and G_2, phases of the cell cycle. Here we report that such a two stage cell cycle arrest, reproducedly occurs in normal rodent embryonic fibroblasts upon reaching *in vitro* senescence, with the accumulation in the G_2 phase exceeding 35% of the total cell number. Additionally Immunofluorescence, and FACS, analyses show that in excess of 90% of the G_2 arrested cells are pre-mitotic. We propose that two stage cell cycle arrest is a mechanism of regulation, distinct from the extensively documented G_0/G_1 arrest of quiescent cells.

E 415 MODULATION OF FIBRONECTIN PRODUCTION IN MELANOCYTIC CELLS BY INTERFERON-γ AND TUMOR NECROSIS FACTOR, Bruce Riser, Raj S. Mitra, Brian J. Nickoloff and James Varani; Department of Pathology, University of Michigan, Ann Arbor, MI 48109. Treatment of normal human foreskin melanocytes and cells from three different human malignant melanomas with interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) inhibited proliferation and induced a change in cell morphology. The treated cells became flattened and polygonal in shape while the untreated cells were bipolar. Treatment of the cells with either cytokine individually had minimal effects. Combined treatment with IFN-γ and TNF-α dramatically increased fibronectin biosynthesis while having only minimal effects on synthesis of two other extracellular matrix components (i.e., laminin and thrombospondin). Treatment with the cytokines individually had much less effect on fibronectin production. Melanocytes and melanoma cells plated onto fibronectin-coated dishes but not onto laminin- or thrombospondin-coated dishes had the morphological appearance of IFN-γ/TNF-α treated cells and the inclusion of antibodies to fibronectin but not laminin partially inhibited the responses of the cells to IFN-γ/TNF-α. Cells which failed to demonstrate increased fibronectin production in response to IFN-γ/TNF-α were isolated from a fourth melanoma. These cells did not show altered morphology or decreased proliferation in the presence of the two cytokines. Taken together, these data suggest that the modulation of melanocyte/melanoma cell behavior by combined treatment with IFN-γ and TNF-α is in part related to alterations in fibronectin production.

E 416 **CHARACTERIZATION OF THE PLATELET-DERIVED GROWTH FACTOR A-CHAIN PROMOTER REGION.** **Fredrik Rorsman & Christer Betsholtz, Dept. of Pathology, University Hospital, S-751 85 Uppsala, Sweden.**

We have structurally characterized the human PDGF A-chain gene and identified a putative promoter region. A TATA box surrounded by GC-rich regions containing several Sp 1 binding sites is situated 870 bp upstream of the translation initiation site. Primer extension studies indicated the mRNA CAP site approximately 25 bp downstream of the TATA box. An 850 bp DNA fragment, containing 450 flanking the 5' end of the CAP site and 450 bp of untanslated sequences, was excised and cloned into the pSV2CAT vector. The resulting CAT fusion gene was transfected into various cell lines. CAT activity could be demonstrated in a human melanoma cell line, a human glioblastoma cell line and a human osteosarcoma cell line, whereas no CAT activity was detectable in a human small cell lung cancer cell line. This correlates with the endogenous PDGF A-chain mRNA expression in these particular cell lines and indicates that the expression of the PDGF A-chain gene is, at least in part, regulated at a transcriptional level and that the excised fragment contains (a) sequence(s) important for this regulation.

The PDGF-A mRNA levels increase in human fibroblasts upon exposure to growth factors. Therefore, we have also transfected the CAT fusion gene into normal human fibroblasts, and we are currently testing the inducibilty of CAT activity in these cells. The results from these experiments will also be presented at the meeting.

E 417 PALYTOXIN DOWN MODULATES THE EPIDERMAL GROWTH FACTOR RECEPTOR THROUGH A SODIUM-DEPENDENT PATHWAY,
Elizabeth V. Wattenberg[1], Paul L. McNeil[2], Hirota Fujiki[3] and Marsha Rich Rosner[1], Ben May Institute, University of Chicago, Chicago, IL 60637[1], Department of Anatomy and Cellular Biology, Harvard Medical School, Boston, MA 02115[2], Cancer Prevention Division, National Cancer Center Research Institute, Tokyo, JAPAN[3]
Palytoxin is a potent marine toxin and mouse skin tumor promoter that is able to act on a wide variety of systems. We have studied the mechanism of palytoxin action in the context of growth control by analyzing the effect of palytoxin on the epidermal growth factor (EGF) receptor in murine fibroblasts. Our results indicate that picamolar levels of palytoxin are able to down modulate the EGF receptor by reducing the number of EGF binding sites. The mechanism of palytoxin action differs from that of TPA-type tumor promoters in several respects, including kinetics, dose-response, and the fact that it is not dependent upon protein kinase C. Further, under our conditions, palytoxin action is sodium-dependent rather than calcium dependent, and palytoxin causes sodium influx with a dose-response that parallels the effects on EGF binding. These results suggest that palytoxin is able to down modulate the EGF receptor through a novel mechanism involving the activation or formation of a sodium pump or channel.

E 418 **Rapid phosphorylation of the L-*myc* protein induced by phorbol ester tumor promoters and serum.**

Kalle Saksela, Tomi P. Mäkelä, Gerard Evan* and Kari Alitalo,
Department of Virology, University of Helsinki, Finland, and *I.C.R.F.L., London, UK.

We have examined posttranslational modification of the L-*myc* protein using polyclonal and monoclonal antibodies against a peptide well conserved in the amino acid sequences of the c-*myc*, N-*myc* and L-*myc* proteins. These antibodies precipitate three polypeptides of M_r 60-66 000 from ^{35}S-methionine or ^{32}P-orthophosphate-labelled human small cell lung cancer cell lines expressing amplified L-*myc* genes, but not the other *myc* genes. Treatment of the L-*myc* immunoprecipitates with alkaline phosphatase prior to electrophoresis converts the three methionine-labelled polypeptides into a single band migrating at M_r 59 000, and efficiently removes radioactivity from the ^{32}P-labelled L-*myc* protein, suggesting that, in contrast to the c-*myc* and N-*myc* proteins, the L-*myc* polypeptide heterogeneity is due to differential phosphorylation of a common precursor. When the phorbol ester 12-O-tetradecanoyl-phorbol-13-acetate (TPA) or serum is added to cultures of U-1690 cells the M_r 66 000 polypeptide is rapidly enriched while the M_r 60 000 form is decreased in the L-*myc* immunoprecipitates. This effect is correlated with the ability of phorbol ester and diacylglycerol analogues to activate protein kinase C. The TPA-induced phosphorylation of the L-*myc* protein occurs in a protein synthesis-independent manner as it is not inhibited by cycloheximide or anisomycin. Our data indicate that the phosphorylation of the L-*myc* nuclear oncoprotein is modulated in response to TPA via a rapid signal transduction system involving protein kinase C. This mechanism could play an important role in the response of lung cells to e.g. bombesin-related growth factors.

E 419 A REGULATORY LOW DENSITY LIPOPROTEIN RECEPTOR-DEPENDENT PATHWAY OF EICOSANOID SYNTHESIS IN PLATELET-DERIVED GROWTH FACTOR-STIMULATED 3T3 FIBROBLASTS.
Peter Salbach[+], Johannes Schenkel[+], Matthias Goerig[+], John A.Glomset[++], and Andreas J.R. Habenicht[+]; [+]University of Heidelberg, School of Medicine, Dept. of Internal Medicine, Bergheimer Str. 58, 6900 Heidelberg, F.R.G.; [++]Howard Hughes Medical Institute Laboratory SL-15 and Regional Primate Research Center SJ-50, University of Washington Seattle, Seattle, WA 98195, USA.
It is well established that the low density lipoprotein (LDL) pathway functions to maintain a constant concentration of cellular cholesterol, but LDL effects that are unrelated to cholesterol metabolism have not been studied in great detail. We have used platelet-derived growth factor (PDGF) stimulated fibroblasts as model systems to investigate mechanisms of LDL-dependent eicosanoid synthesis. PDGF-stimulated but not quiescent cells formed radiolabeled prostacyclin and PGE_2 upon incubation with LDL that had been reconstituted wth cholesteryl-(1-^{14}C) arachidonate. In contrast, cells that had been preincubated with excess unlabeled LDL or chloroquine did not produce significant amounts of labeled eicosanoids.Incubation of PDGF-stimulated cells with LDL or arachidonic acid but not incubation with cholesterol led to a time- and concentration-dependent inactivation of PGH synthase, the key enzyme in prostaglandin synthesis. After removal of LDL, PGH synthase activity recovered within 6 h and recovery required translational activity. These results demonstrate that arachidonic acid in LDL is metabolized to form prostacyclin and PGE_2 in PDGF-stimulated fibroblasts and suggest that the arachidonic acid in LDL mediates the inhibition of PGH synthase activity.

E 420 MNC-DERIVED GROWTH STIMULATORY ACTIVITY FOR HUMAN TUMOR CELL LINES. George Sandru, Peter Veraguth, Radiobiology Laboratory, Department of Radiotherapy, University of Berne, Inselspital, 3010 Berne, Switzerland. Serum-free supernatants of peripheral blood mononuclear cell cultures significantly stimulated H3-thymidine incorporation of human hematopoietic and nonhematopoietic tumor cell lines. For assay we used a low number of tumor cells per well and medium enriched with dithiothreitol-treated fetal calf serum. The growth stimulatory activity was detected in the supernatant of peripheral blood mononuclear cell cultures within the first 24 h and there after decreased. Treatment of mononuclear cells with OKT3 monoclonal antibodies and rabbit complement decreased only moderately the factor production while treatment with anti-Leu M2 and rabbit complement decreased it significantly. Irradiation or pulse exposure to puromycin of mononuclear cells before performing the cultures increased production of the growth stimulatory activity. Supernatants of concanavalin A-stimulated MNC cultures enhanced nonsignificantly H3-thymidine incorporation by tumor cell cultures unless antibodies against tumor necrosis factor-α and γ-interferon were added to the supernatants. Growth stimulatory activity was heat inactivated partially at 60°C and totally at 80°C. It was also abolished by treatment with dithiothreitol as well as at pH 2.5 within 2 h and partially lost by dialysation.

E 421 BCNU-RESISTANT HUMAN GLIOMA CELLS EXHIBIT AUTOCRINE REGULATION OF PLATELET-DERIVED GROWTH FACTOR. A.C. Scheck, P.L. Moots, B.M. Mehta, S.A.D. Ebrahim, and J.R. Shapiro. Dept. of Neurology, Memorial Sloan-Kettering Cancer Center, NY, NY 10021. Human malignant glioma consists of cells that are phenotypically and genotypically heterogeneous. We have described a subpopulation of cells in these tumors with a specific karyotypic over-representation of chromosomes 7 and 22 that are resistant to BCNU in vitro. The A- and B-chains of platelet-derived growth factor (PDGF) have been mapped to these chromosomes. Growth, receptor binding and phosphorylation studies demonstrated that these cells produce a PDGF-like peptide. DNA hybridization analyses demonstrated the presence of multiple copies of the genes encoding the PDGF-A and -B chains in the BCNU-resistant compared with BCNU-sensitive cells or normal glia. To determine if selection for such cells occurs following in vivo BCNU treatment, cells were isolated from freshly resected tumors that recurred after the patients received BCNU therapy. Karyotypic, biochemical and molecular analyses identified the recurrent tumor cells as the same subpopulation of cells isolated in the in vitro assay. This subpopulation was present in some but not all areas of the primary tumor as shown by regional analyses of two gliomas removed almost in toto. These cells had over-representation of chromosomes 7 and 22, and an increased PDGF gene copy number in some, but not all regions. To determine if such cells secreted a PDGF-like peptide, they were grown in media containing 1% fetal calf serum (FCS). Northern blot analysis suggested that this subpopulation of cells over-expresses the PDGF-A chain gene. Thus, aberrant expression of PDGF may be a marker for resistant cells in human gliomas.

E 422 RECONSTITUTED BASEMENT MEMBRANE ENHANCES NEURITE OUTGROWTH IN PC-12 CELLS INDUCED BY NERVE GROWTH FACTOR, Martin A. Schwarz and David L. Emerson, Depts. of Cell Culture Research and Tumor Biology, Schering Research, Bloomfield, N.J. 07003

Rat Pheochromocytoma cells (PC-12 cells) respond to nerve growth factor (NGF) by elaborating neurites. PC-12 cells are plated on dishes coated with type I collagen (COL), fibronectin (FN), or laminin (LMN) before stimulation with exogenous NGF. To study the effect of a more complex substratum on neurite outgrowth PC-12 cells were entrapped in reconstituted basement membrane prepared from Engelbreth Holm Swarm tumors (EHS-BM). NGF elicited neurite outgrowth in cells plated on COL, FN , or LMN within 48-72 hours, in contrast cells plated in EHS-BM respond within 24 hours. Cells on COL or FN required a minimal dose of 50ng/ml NGF to achieve the same effect as cells in EHS-BM at 1ng/ml. Neurites in cells in EHS-BM were significantly longer and more extensively branched. Finally, neurite outgrowth typically requires further addition of NGF otherwise neurites retracted within 7 days. Neurites in cells plated in EHS-BM remained intact up to 21 days following a single administration of NGF. Thus, from these obseravtions it appears that EHS-BM may act in concert with NGF to enhance and sustain neurite ougrowth by providing a more physiological environment for neuronal cell differentiation to occur.

E 423 BINDING OF TGF-ß TO CELL SURFACE PROTEINS VARIES WITH CELL TYPE, Patricia R. Segarini, David M. Rosen and Saeid M. Seyedin. Celtrix Laboratories, Collagen Corporation, 2500 Faber Place, Palo Alto, CA 94303.

Transforming growth factor-ßs (TGF-ß1 and TGF-ß2) bind to several different cell surface proteins, including a high M_r proteoglycan (Segarini and Seyedin, (1988) J. Biol Chem. 263, 8366-8370). We found that on primary and early passage cultures of fibroblasts, chondroblasts, and osteoblasts TGF-ß1 and TGF-ß2 bind to both the high M_r proteoglycan and to lower M_r components, whereas on epithelial, endothelial, and lymphoid-derived cells TGF-ß1 and TGF-ß2 only bind to the lower M_r species. With cell lines, this distinction is lost. Further analysis indicated that binding to the high M_r proteoglycan is not necessary for TGF-ß induced regulation of DNA, collagen and fibronectin synthesis, change in cell morphology, or reorganization of the actin cytoskeleton. Based on the relationship between TGF-ß binding and function, we propose that the lower M_r components are the active receptors mediating these events, whereas the function of the proteoglycan remains unknown.

E 424 A NOVEL POLYPHOSPHOINOSITIDE PATHWAY ACTIVATED BY TRANSFORMATION AND GROWTH FACTOR STIMULATION, L.A. Serunian, K.A. Auger, and L.C. Cantley, Department of Physiology, Tufts University School of Medicine, Boston, MA. 02111.

Our laboratory has characterized a unique phosphatidylinositol (PI)-3' kinase activity that is tightly associated with protein tyrosine kinases (e.g. polyoma middle t/pp60$^{c\text{-}src}$,) and with the platelet-derived growth factor (PDGF) receptor. Unlike previously described PI kinases which phosphorylate the D-4 position of the inositol ring, the PI-3' kinase phosphorylates the D-3 position to produce PI-3-P which is not in the pathway for generating Ins-1,4,5-P_3. Using immunoprecipitation assays, we have shown that this kinase phosphorylates two other lipids--PI-4-P, to generate a unique PI-3,4-P_2 product, and PI-4,5-P_2, to produce a PIP_3 species, but does not phosphorylate soluble inositol phosphates. The PI-3-P, PI-3,4-P_2, and PIP_3 polyphosphoinositides can be separated from PI-4-P and PI-4,5-P_2 by HPLC analysis.

The novel lipids generated *in vitro* also appear *in vivo* following oncogenic or PDGF stimulation. The correlation between transformation and increased levels of these lipids exists in several transformed cell lines tested. In quiescent Balb/3T3 fibroblasts, the highly phosphorylated novel lipids are virtually undetectable; within 1 minute of PDGF addition, the PI-3,4-P_2 and PIP_3 species appear. Although the quantities of PI-3-P, PI-3,4-P_2 and PIP_3 are small compared with the quantities of the lipids in the conventional PI pathway, changes in the amounts of the novel lipids are dramatic and clearly detectable. Thus, the activation of this new polyphosphoinositide pathway may play a pivotal role in transformation by certain oncogenes and in cellular responses to growth factors like PDGF.

E 425 **Incoordinate regulation of growth factor and oncogene mRNA during differentiation of HL60 cells.** R J Shaw, R A F Clark, S H Benedict. National Jewish Center for Immunology and Respiratory Medicine, Denver, Co 80206.

The promyelocytic cell line HL60 differentiates to a macrophage-like phenotype when stimulated by TPA. These cells served as a model to study the intra-nuclear events of the macrophage differentiation process. The two stimuli, TPA and dBcAMP, arrested proliferation, whereas, only TPA caused macrophage-like morphologic changes. Both agents caused an increase in the level of mRNA encoding the differentiation related oncogene c-fos. This was maximal between 30 and 60 min following TPA, and between 60 and 120 min for dBcAMP. TPA stimulation caused an increase in the mRNA encoding Platelet Derived Growth Factor (PDGF), Fibroblast Growth Factor (FGF), and Transforming Growth Factor beta (TGF-beta), at 24-48 hours. In contrast, stimulation with dBcAMP resulted in an increase in PDGF and FGF mRNA without a concomitant increase in TGF-beta mRNA. These data suggest that, in HL60 cells, TPA and dBcAMP stimulate an increase in similar but not identical oncogene and growth factor mRNAs, and thus that the increase in these specific mRNAs is not subject to coordinate regulation.

E 426 RESPONSE TO AND PRODUCTION OF aFGF, bFGF AND TGF-α BY CULTURED NORMAL HUMAN SKIN CELLS, Gary D. Shipley, Paul W. Cook, Mark R. Pittelkow and Robert J. Coffey, Jr.; Oregon Health Sciences University, Portland, OR; Mayo Clinic, Rochester, MN (MRP); and Vanderbilt Univ., Nashville, TN (RJC). Fibroblasts, keratinocytes and melanocytes can be grown and studied in defined, serum-free medium. Our recent studies have focused on the ability of acidic and basic fibroblast growth factor (aFGF and bFGF) and transforming growth factor type-α (TGF-α)/epidermal growth factor (EGF) to control the rate of proliferation in these cells. In addition, we have studied the expression of the mRNAs for these growth factors in the same cells to determine possible paracrine or autocrine relationships. Distinct patterns have emerged. All three cell types are stimulated to proliferate by aFGF and bFGF. For fibroblasts and melanocytes, bFGF appears to the the most potent mitogen. In low density keratinocyte cultures, however, aFGF was more potent than bFGF. The addition of heparin (10 μg/ml) enhanced the activity of aFGF and to a lesser extent bFGF when tested on fibroblasts and melanocytes. In contrast, heparin dramatically reduced the ability of bFGF but not aFGF to stimulate the proliferation of keratinocytes. TGF-α/EGF stimulated the proliferation of fibroblasts and keratinocytes but had no effect in melanocyte cultures. Heparin had no effect on TGF-α/EGF-stimulated growth. We examined these cells for the expression of growth factor mRNA. mRNA coding for aFGF and bFGF are present in fibroblasts and the abundance of these mRNAs can be regulated by the addition of serum. The levels of FGF mRNAs in growing cultures of keratinocytes are very low to undetectable. The addition of TGF-β to keratinocyte cultures causes an increase in bFGF but not aFGF or TGF-α mRNA. TGF-α mRNA is present in keratinocytes and is induced by serum and TGF-α/EGF but is not present in fibroblasts under similar conditions. To date we have been unable to detect mRNA for these growth factors in melanocyte cultures. Our results suggest that distinct patterns of responsiveness and gene expression occur in normal cell types derived from the same tissue. Supported by CA42409 (GDS), CA46413 (RJC) and the Mayo Fnd. (MRP).

E 427 **EGF induces transduction of mitogenic signals in cells expressing a chimeric EGF-receptor/*neu* proto-oncogene** Lea Sistonen, Laura Lehtola, Heikki Lehväslaiho, Erkki Hölttä* and Kari Alitalo, Departments of Virology and Pathology*, University of Helsinki, 00290 Helsinki, Finland.

A chimeric cDNA encoding epidermal growth factor receptor (EGF-R) extracellular, transmembrane and protein kinase C-substrate domains linked to the intracellular tyrosine kinase and carboxyl terminal domains of the rat *neu* receptor was constructed and expressed in NIH/3T3 cells. EGF stimulation of the receptor-expressing but not control cells caused rapid and delayed morphological changes in the cells followed by increased DNA synthesis and cell proliferation. EGF bound to the cells was rapidly internalized in a complex with the EGF-R/*neu* protein, as shown by [125I]EGF binding studies and loss of EGF antigens from the cell surface. The movement of the EGF-R/*neu* protein was followed with indirect immunofluorescence into a vesicular intracellular compartment. Metabolic labeling and pulse-chase experiments indicated that the receptor was degraded soon after its internalization. We also analysed the transduction of rapid and delayed signals for gene activation upon stimulation of the chimeric receptor. Serum-starved NIH/3T3 cells expressing various amounts of the EGF-R/*neu* receptor were stimulated by EGF and the activity of ornithine decarboxylase (ODC) was measured as a function of time of stimulation. At 24 h the enzyme activity was increased 100- to 1000-fold in different clones depending on the number of the chimeric receptors on the cell surface while the ODC mRNA was induced maximally 2-10-fold after a 4-hour EGF stimulation. The stimulation of ODC expression was preceded by a transcriptional activation of *jun* genes and synthesis of the AP-1/JUN transcriptional factor. We conclude that the chimeric receptor is capable of transducing an EGF-induced signal for growth stimulation and the associated enzymatic and transcriptional changes.

E 428 A GTP-BINDING PROTEIN IS INVOLVED IN A LATE EVENT DURING ENDOGENOUS GANGLIOSIDE-MODULATED CELLULAR PROLIFERATION, Sarah Spiegel, Dept. of Biochemistry, Georgetown University Medical Center, Washington, DC 20007. The B subunit of cholera toxin, a protein which binds specifically to ganglioside GM1 on the cell surface, stimulates DNA synthesis in quiescent Swiss 3T3 fibroblasts. Pertussis toxin (PT) pretreatment markedly inhibits B subunit-induced DNA synthesis. The inhibitory effects were observed even in the presence of insulin which greatly potentiates the mitogenic response to the B subunit. The dose-response for PT-induced inhibition of DNA synthesis correlated closely with the dose-response for ADP-ribosylation of a 41 kD membrane protein, suggesting the involvement of a GTP-binding protein (Gi) in mitogenesis induced via crosslinking of endogenous gangliosides. PT also inhibited the mitogenic response to unfractionated fetal calf serum and to bombesin in the absence or presence of insulin in an identical concentration range. The inhibitory effect of PT was clearly unrelated to any effects on known G proteins coupled to adenylate cyclase or phospholipase C. PT-induced inhibition could still be observed even when the toxin was added as late as 6 hours after addition of the growth promoting agents. This suggests the involvement of a GTP-binding protein in a late step of the B subunit- and bombesin-mediated pathways of mitogenesis. The possibility that other growth factors bypass this pathway is shown by their lack of sensitivity to pertussis toxin.

E 429 TRANSCRIPTION OF INSULIN-LIKE GROWTH FACTORS IN HUMAN BREAST CANCER, Michael D. Strathearn and E. Martin Spencer, Laboratory of Growth and Development, Children's Hospital of San Francisco, San Francisco, CA 94119

Recent reports in the literature indicate that there is autocrine and paracrine growth regulation of human breast cancer. Human breast cancer cell lines produce a potent mitogen, insulin-like growth factor-I (IGF-I, somatomedin-C) suggesting, but not establishing, an autocrine mechanism, that may contribute to the malignant biologic behavior of these tumors. Estrogens may act by allowing breast cancers to overcome growth-inhibitory agents in their environment. Human breast cancer cells have shown a two- to three-fold increase in secreted IGF-I production with estrogen treatment. This is consistent with a role for IGF-I as a mediator for estrogen induction of breast cancer and progression of breast cancer to more aggressive forms. Since mammary tissues have receptors for IGFs, transcription of IGFs by breast neoplasms may result in autostimulation of growth.

The objective of this study was to determine the level of insulin-like growth factor (IGF)-I mRNA levels in human breast cancers in comparison to normal breast levels in tissue samples. In preliminary results, all 12 ductal breast carcinomas examined showed IGF-I production by immunohistochemical staining. Levels of mRNA isolated from the tumors were quantified by slot-blot analysis; results suggest that there is a regulation of IGF-I in breast cancers. As there are several size classes of IGF mRNAs, Northern blots were used to determine their distribution and whether individual species are differentially regulated. IGF mRNAs are distributed in different size classes of mRNA that correlate with tumor type, suggesting that there might be a unique control mechanism in breast cancer cells that regulates the processing of IGF-I mRNA. Breast cancer cell lines have been used to further explore the regulation of IGF gene transcription. The level of mRNA will be correlated to estrogen responsivity and cell line; sensitive lines probed with estrogen have been used to determine the effect on IGF gene transcription.

E 430 ALTERNATIVE SPLICING OF THE PDGF A CHAIN TRANSCRIPT OCCURS IN NORMAL AS WELL AS TUMOR CELLS AND IS CONSERVED AMONG MAMMALIAN SPECIES, Viveka Svensson, Brona Matoskova, Fredrik Rorsman and Christer Betsholtz. Department of Pathology, University Hospital, S-75185 Uppsala, Sweden.

Alternative splicing of the human platelet-derived growth factor A chain (PDGF-A) transcript results in the presence or absence of a 69-base-pair (bp) segment in the mature messenger RNA molecule. This has previously been demonstrated only in a human glioma cell line and has therefore been suggested to constitute a tumor-specific phenomenon. The 69-bp region represents a separate exon in the A chain gene, exon 6. Using polymerase chain reaction (PCR) methodology, we here demonstrate that this type of alternative splicing occurs, not only in human tumor cells but also, in contrast to previous reports, in a number normal human cell types, including endothelial cells. Alternative usage of a DNA sequence homologous to human exon 6 was detected also in normal tissues from other mammalian species. The evolutionary conservation indicate that a specific and indispensible, but so far unknown, function is associated with exon 6 of the PDGF A chain gene.

E 430A FUNCTIONAL SIGNIFICANCE OF ALTERED GLYCOSYLATION IN EXPRESSION OF MALIGNANT POTENTIAL OF TUMOR CELLS - VARIANT RECEPTORS, Tien-wen Tao, Orhan Oz, Hung Pham, Division of Nuclear Medicine, Stanford University School of Medicine, Stanford, CA 94305

A wheat-germ-agglutinin resistant mutant (WGA-R) of mouse B16 melanoma cells showed dramatically reduced malignant potential including decreased tumorigenicity and decreased metastatic capacity. The biochemical phenotype of the mutant is altered oligosaccharide structure affecting only the N-linked oligosaccharides. Our working hypothesis is that this post-translational modification of protein structures with respect to glycosylation alters the structure and the function of a variety of molecules which mediate different biological processes, such as adhesion receptors and receptors for growth factors. Fibronectin receptor (FNR) and receptors for IGF I and II (IGFR) were studied. FNRs were isolated from detergent extracts of B16 and WGA-R cells surface-labeled with ^{125}I using goat anti-gp140 (generous gift of Dr. P. Brown) and analyzed by SDS-polyacrylamide gel electrophoresis. FNR derived from the mutant showed a faster electrophoretic mobility than that derived from the parental cells. The mobility difference was abolished following deglycosylation using N-glycanase or trifluoromethanesulfonic acid indicating that the structural alteration of the variant FNR resides in its glycan component. Associated with the structural alteration of the FNR, the mutant cells spread and adhered poorly on the cell-binding domain of fibronectin, events mediated by the FNR. The IGFRs were analyzed by cross-linking of ^{125}I-IGFs to cells followed by gel electrophoresis. As with the FNR, the receptors derived from mutant cells also showed faster electrophoretic mobility suggesting the presence of altered oligosaccharide structure.

E 431 REGULATION OF NUCLEAR PROTO-ONCOGENE EXPRESSION BY SERUM IN NON-SENESCENT MOUSE EMBRYO CELL CULTURES, Dennis Templeton, Lorene Lanier, and Robert A. Weinberg, Whitehead Institute, Nine Cambridge Center, Cambridge Mass 02142

Primary mouse embryo cells grown in serum-containing medium undergo a limited number of cell divisions and then cease dividing, a phenomenon loosely referred to as senescence. Barnes *et al.* (Science 236:200-202, 1987) demonstrated that mouse embryo cells grown in defined medium without serum supplementation undergo continuous cell division, apparently being non-senescent, and that even at late passage number these cultures remain diploid. These cells (referred to as SFMEs) respond to subsequent serum supplementation by a rapid cessation of cell division.

We have confirmed the observations of Barnes' group and have begun to examine the transcriptional response to serum stimulation in comparison to serum-grown primary cells or to established mouse fibroblasts. Serum stimulated SFME cultures express c-fos and egr-1 messages to the same level and with similar kinetics to serum-grown cells. In contrast to the response of serum-grown cells, serum stimulated SFME's do not exhibit elevated levels of c-myc. We are currently examining the response of other known serum-response messages.

In addition, we have infected cultures of SFMEs with recombinant retroviruses which express several nuclear and cytoplasmic oncogenes; the biological changes observed in these cells will be discussed.

E 432 MODULATION OF OVARIAN CELL FUNCTION BY MACROPHAGE-DERIVED ANGIOGENIC FACTORS, Matsuo Ui, Naoya Emoto, Jean-Jacques Feige, and Andrew Baird, Laboratories for Neuroendocrinology, Salk Institute, 10010 N. Torrey Pines Road, La Jolla, CA 92037

Macrophages have been linked to all processes that involve tissue repair and remodeling. As such, many of the cytokines that are produced by macrophages have been found to contribute to these processes. One of the physiological functions of macrophages is to participate in the tissue remodeling associated with normal reproductive function. For this reason, we have been investigating the role of various macrophage-derived cytokines in the ovary. Tumour necrosis factor-α (TNFα), transforming growth factor-β (TGFβ), and basic fibroblast growth factor (FGF) are cases in point. These three proteins are angiogenic and modify granulosa cell function. Their effects, however, are significantly different. TNFα and FGF can inhibit aromatase activity (the production of estrogens) in these cells and prevent the induction of receptors for luteinizing hormone (LH) by follicle stimulating hormone (FSH). While TNFα inhibits progesterone synthesis, basic FGF can increase the response to follicle stimulating hormone (FSH). In contrast, TGFβ can enhance the production of estrogens by granulosa cells when it is tested in the presence of FSH. This activity is also shared by the structurally related homodimer TGFβ_2 and the heterodimer TGF$\beta_{1\text{-}2}$. The treatment of macrophages and capillary endothelial cells with TGFβ increases the amount of basic FGF mRNA as detected by Northern analyses, demonstrating that these factors can cross-regulate their expression as well as have differential effects on their common target cell function. Thus, the results suggest that the activities of these angiogenic factors in the ovary are closely interrelated and that their regulation at the cellular level will ultimately modulate the response of the target cell.

E 433 INDUCTION OF THE EPITHELIUM TO MESENCHYME TRANSITION IN A TUMOR CELL LINE. Vallés, A.M., Boyer, B., Tucker, G.C., Gavrilovic, J., Jouanneau, J.and Thiery, J.P. Laboratoire de Physiopathologie du Développement, Ecole Normale Supérieure, 75230 Paris Cedex 05, France. The epithelial to mesenchymal transition (EMT) is a basic process in morphogenesis and most likely occurs during cancer invasion and metastasis. We have been investigating the role of extracellular matrix molecules (ECM) and soluble factors in inducing this event using the rat bladder carcinoma cell line, NBT-II as a model system. This cell line when cultured in basic medium (DMEM plus 10% FCS) has a typical epithelial phenotype and does not translocate. However, in the presence of different types of collagen as substratum, the cells lose their epithelial characteristics and acquire properties typical of mesenchymal cells: they lose their intercellular junctions as evidenced from anti-desmosomal components immunocytochemistry, undergo cytoskeletal changes and gradually acquire a fibroblastic appearance and motile behavior. Thus, this system provides the potential to study the interactions between ECM components and soluble factors with tumor cells and their specific role in carcinoma metastasis.

E 434 NEU GENE AMPLIFICATION AND PROTEIN OVEREXPRESSION IN COMEDO TYPE DUCTAL CARCINOMA IN SITU. Marc van de Vijver, Johannes Peterse, Els Wagenaar, Wolter Mooi and Roel Nusse, Departments of Molecular Biology and Pathology, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, Netherlands.
Neu gene amplification and protein overexpression is present in 15-30 % of human invasive breast carcinomas. We have raised monoclonal antibodies against the human neu protein. Using immunohistochemistry on paraffin sections, these monoclonal antibodies can detect neu protein overexpression. Neu protein overexpression is visible as a strong membrane staining of tumor cells.
In a large series of ductal carcinomas in situ, almost all comedo type ductal carcinomas in situ show neu protein overexpression. Ductal carcinomas in situ of a papillary or cribriform histologic type never show neu protein overexpression. In the comedo type ductal carcinomas in situ positive for neu membrane staining analyzed, neu gene amplification was found to be present. We conclude that neu protein overexpression, probably always as a result of neu gene amplification, defines a distinct group of ductal carcinomas in situ. The implication of our findings is that neu gene amplification is a requirement for this tumor type to develop.

E 435 TRANSFORMING PROPERTIES AND SUBCELLULAR LOCALIZATION OF abl PROTEIN VARIANTS. Rick Van Etten, Peter K. Jackson, George Q. Daley, and David Baltimore. Whitehead Institute, MIT, Cambridge MA 02142.
By indirect immunofluorescence in transformed fibroblasts, the v-abl protein is in the cytoplasm and at the inner surface of the plasma membrane, with particular affinity for regions of cell-cell contact, the Golgi apparatus, and adhesion plaques. Overexpression of either type I or type IV c-abl is insufficient for transformation of fibroblasts. Overexpressed type IV c-abl protein is found mainly in the nucleus. Deletion of 53 amino acids N-terminal to the kinase region of the type IV c-abl generates a fully transforming protein which has a subcellular localization indistinguishable from v-abl. A number of non-myristoylated abl variants, including the CML-specific P210 bcr/abl protein and a point mutant of v-abl, are evenly distributed through the cytoplasm or in the Golgi. These proteins are unable to transform fibroblasts but retain the ability to transform lymphoid cells in vitro.

E 436 MODULATION OF FIBRONECTIN, FIBRONECTIN RECEPTORS AND CELL-SUBSTRATE ADHESION IN THE TRANSFORMATION AND DIFFERENTIATION OF MURINE TUMOR CELLS, James Varani and Subhas Chakrabarty; Dept. of Pathology, University of Michigan, Ann Arbor, MI 48109 and Dept. of Pharmacology; Baylor College of Medicine, Houston, TX 77030.
Murine cells transformed with 3-methylcholanthrene or with the ras oncogene demonstrate increased growth in monolayer and suspension culture and tumorigenicity in athymic mice. Transformation is accompanied by a loss of surface fibronectin (FN) without a loss of surface laminin. Treatment of the transformed cells with N,N-dimethylformamide (DMF) reverses the transformed phenotype and this is associated with a reacquisition of surface FN. The alternations in FN expression accompanying transformation/differentiation result from changes in both FN synthesis as indicated by metabolic labeling/immunoprecipitation studies and FN receptor expression as indicated by ^{125}I-FN binding to whole cells and cell lysates separated by SDS-PAGE and transferred to nitrocellulose. Accompanying the changes in FN and FN receptor expression are changes in cell-substrate adhesion. Upon transformation, the cells show decreased adhesion to FN- and bovine serum albumin-coated dishes but not to laminin-coated dishes. Changes in cell-substrate adhesion are reversed by treatment with DMF. Taken together, these data indicate that alternations in FN metabolism accompany transformation/differentiation of murine cells and may underlie some of the biological properties that are influenced by transformation and by differentiation.

E 437 INCREASE IN PROTEIN TYROSINE PHOSPHATASE AND KINASE LEVELS DURING DIFFERENTIATION OF 3T3 L1 CELLS Emma Villa Moruzzi, Nicholas K. Tonks, James Sommercorn,Edwin G. Krebs & Edmond H. Fischer,Department of Biochemistry, University of Washington, Seattle, WA 98195. Protein tyrosine phosphatases (PTPases) partitioned between the soluble (extracted with low ionic strength aqueous buffer) and particulate fractions (extracted with the same buffer + 0.5% Triton X-100-0.2 M NaCl) of 3T3-L1 cells in a ratio of 15-85. Protein tyrosine kinases (PTK) distributed in a ratio of 30-70 between these two fractions. Differentiation into adipocytes was induced by dexamethasone and 3 isobutyl 1 methylxanthine. Both soluble and particulate PTPases increased 3-4 fold within 2 days of induction with most of the increase occurring within one day. Due to the lower day zero activity, the soluble PTPases showed a greater fold increase. Both soluble and particulate activities were stimulated by EDTA. The increase in PTPase activity was paralleled by a 2-4 fold increase in PTK, which was more pronounced in the soluble fraction. All the effects on PTPases and on soluble PTK occurred faster than the increase in cell number and also preceded the appearance of elevated insulin binding. Some limited characterization of the soluble and particulate PTPases will be presented.

E 438 QUANTITATIVE RESOLUTION OF RECYCLING AND NONRECYCLING CYTOKINE RECEPTORS IN TUMOR CELLS BY COMPUTER MODELING. S. Vuk-Pavlović, Ž. Bajzer, A.C. Myers. Oncology Research/Biochemistry and Molecular Biology, Mayo Foundation, Rochester, MN 55905. We measured the time-dependent concentration changes of human interferon-α2a (IFN) and human tumor necrosis factor-α (TNF) at the plasma membrane and internalized by human lung alveolar carcinoma A549 cells in the presence of excess free ligand. The patterns of these concentration changes for two ligands were significantly different. On the basis of qualitative evidence, we modified our compartmental kinetic model encompassing receptor synthesis and receptor loss (Myers et al., J. Biol. Chem., 262:6494; 1987) to include receptor recycling. We solved the equations of three variants of the recycling model analytically. All parameters (rate constants) were identifiable when the data sets consisted of time-resolved concentrations of IFN and TNF at the cell surface and internalized by cells. By the least-square fitting we derived the best-fit values of the first-order rate constants for internalization of the ligand-receptor complex, receptor recycling, turnover of free receptors, elimination of the ligand from cells and the rate of insertion of free receptors into the membrane. We found that the best fit to IFN-data was obtained without including the term for recycling receptors to the membrane; the simplest model including receptor recycling was necessary and sufficient for the fit to TNF-data. These results demonstrate that the contribution of receptor recycling to the ligand/receptor metabolism can be quantitated by use of compartmental modeling. In A549 cells, receptor recycling does not contribute to the kinetics of Type I IFN-receptor; recycling contributes significantly to endocytosis mediated by the TNF-receptor. Supported in part by CA 45312, NIH, DHHS.

E 439 GENERATION OF POTENT GASTRIN RELEASING PEPTIDE ANTAGONISTS BY C-TERMINAL MODIFICATION OF N-ACETYL-GRP-20-27, J.W. Wallen, D.C. Heimbrook, W.S. Saari, N.L. Balishin, A. Friedman, M.W. Riemen, D.M. Kiefer, N.S. Rotberg and A. Oliff, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.
Gastrin releasing peptide (GRP) is a 27 amino acid peptide hormone which is homologous to the amphibian peptide bombesin. GRP serves a variety of physiological functions, and has been implicated as an autocrine factor in the growth regulation of small cell lung cancer (SCLC) cells. We have developed a series of potent GRP antagonists by modification of the C-terminus of N-acetyl- GRP 20-27. These antagonists exhibit IC_{50}'s in the 10^{-8} to 10^{-9} molar range in a competitive binding inhibition assay. One of these compounds was further shown to block GRP-stimulated mitogenesis in Swiss 3T3 mouse fibroblasts, inhibit GRP-dependent release of gastrin in vivo, and block GRP-induced elevation of $]Ca^{2+}]_i$ in H345 SCLC cells. These results demonstrate that while residues 20-27 of GRP influence binding of the parent peptide to its receptor, the C-terminal amino acid is primarily responsible for triggering the subsequent biological response.

E 440 BOVINE AORTIC SMOOTH MUSCLE CELLS: ROLE OF DIFFERENT GROWTH FACTORS FOR CELLULAR REPLICATION, Herbert A. Weich, Judah Folkman, Children's Hospital and Harvard Medical School,Boston, MA 02115

A serum-free assay has been established for studying the role of different polypeptide growth factors during the regulation of bovine aortic smooth muscle cells (baSMC) growth. Overconfluent, quiescent cell cultures were stimulated by different growth factors, alone or in combination, and DNA-synthesis was measured by incorporation of ^{3}H-thymidine. Individual administration of bFGF, aFGF, PDGF, IGF-1 and EGF at 10 ng/ml and insulin at high concentrations (5 ug/ml) increase DNA-synthetic rates approximately 8- to 18-fold in relation to controls, whereas TGF-beta, rat IGF-2 and insulin at low concentrations (10 ng/ml) had no effect. When combinations of growth factors were studied we found that while EGF and FGF had an additive effect, other combinations were synergistic. For example, basic FGF together with IGF-1 at physiological concentrations or with insulin at high concentrations were highly synergistic and produce ^{3}H-thymidine incorporation levels that were similar to those produced with 10% calf serum.
To study the role of IGF-1 for baSMCs, ^{125}I-labeled IGF-1 was used for receptor analysis. Scatchard analysis showed, that baSMCs have 6200 receptors per cell with a K_D of 2.9 nM. Cultivation of these cells over 5 days in def. medium without insulin or IGF-peptides showed that the number of IGF-1 receptors was upregulated to 14500 receptors per cell, without altering receptor affinity. Chemical crosslinking experiments with ^{125}I-IGF-1 in combination with SDS-PAGE under reducing conditions demonstrated a major band of Mr 130 kd. The receptor complex formation was inhibited by IGF-1, human IGF-2 or high concentrations of bovine insulin, whereas heterologous growth factors had no effect.

E 441 BIOSYNTHESIS AND PROCESSING OF THE RECEPTOR FOR PDGF IN A HUMAN TERATOCARCINOMA CELL LINE PRODUCING A PDGF-LIKE GROWTH FACTOR, Sjerp M. Weima, Marga A. van Rooijen, Christine L. Mummery, Alie Feijen, Siegfried de Laat and Everardus J.J. van Zoelen. Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands. The human teratocarcinoma cell line Tera 2 clone 13, T2 cl-13, can be induced to differentiate in-vitro into endodermal and neuroectodermal cell types by retinoic acid, (RA). Undifferentiated cells, (EC cells), are essentially growth factor independent and rapidly proliferating whereas RA treated cells have a prolonged generation time and a limmited lifespan. In a previous study we have shown that T2 cl-13 cells produce significant amounts of a PDGF like protein, most likely a homodimer of polypeptides coded for by the PDGF-A chain gene. This PDGF like molecule is a strong mitogen for 3T3 and NRK fibroblasts. Upon RA induced differentiation the PDGF-A gene, and thus PDGF production is decreased to hardly detectable levels, (Weima et al, in press). In the present studie we describe that both T2 cl-13 EC and RA treated cells express the PDGF receptor gene. Pulse-chase experiments on metabolicaly labelled EC and RA treated cells revealed that, endogenous as well as high dosis of recombinant PDGF-AA did not accelerate PDGF receptor turnover. In contrast, PDGF-AB and PDGF-BB added to the culture dishes rapidly increased receptor breakdown in EC and RA treated cells. These results indicate that PDGF-AA does not bind to the receptor type which binds PDGF-AB and BB. Therefore autocrine growth stimulatory effects of PDGF-AA in T2 cl-13 EC cells and/or mitogenic activity towards other cells should be mediated through a yet unidentified second type of PDGF receptor.

E 442 HUMAN PLATELET FACTOR 4 INHIBITS BINDING OF TGF-β_1 TO THE 63 kDa TGF-β_1 RECEPTOR/BINDING PROTEIN. Robert H. Whitson, Wee Ling Wong and Keiichi Itakura, Beckman Research Institute of the City of Hope, Duarte,CA 91010. Acid/ethanol extracts of human platelets were fractionated on a gel filtration column, and the fractions were tested for their ability to compete with ^{125}I-labeled TGF-β_1 for binding to intact cells. Using normal rat kidney (NRK) fibroblasts in the binding assay, a single peak of inhibitory activity which proved to be TGF-β_1 was detected. When the human hepatocellular carcinoma line Hep 3B was used, two inhibitory peaks were observed. One coincided with the TGF-β_1 peak and the other eluted with the low molecular weight fractions. Further purification of the low molecular weight inhibitor on a C_{18} reversed-phase HPLC column yielded a homogeneous protein of ~7,000 kDa. Amino-terminal sequencing gave an unambiguous match in 33 of 35 residues with human platelet factor 4. Identification of the competing material as platelet factor 4 was further supported by the fact that it bound tightly to heparin- sepharose. Affinity cross-linking experiments revealed that platelet factor 4 inhibited TGF-ß binding only to a high affinity 63 kDa TGF-β_1 receptor, the principal TGF-ß binding protein in Hep 3B cells. The major TGF-ß binding protein of NRK cells is a high molecular weight receptor which also binds TGF-β_2; platelet factor 4 does not inhibit TGF-ß binding to this receptor. The biological consequences of the inhibitory activity of platelet factor 4 are not clear.

E 444 REGULATION OF GENE EXPRESSION DURING INDUCTION-DIFFERENTIATION OF HUMAN NEUROBLASTOMA CELLS, Bryan R.G. Williams[1], David Malkin[2],[1], Greg Hannigan[1], Sue Chilton-MacNeil[1],[3] and Herman Yeger[3], Division of Infectious Diseases[1], Hematology/Oncology[2] and Pathology[3], Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8

Neuroblastoma (NB) presents a versatile model for the investigation of molecular mechanisms governing the process of induced and spontaneous tumor cell differentiation. We have established cell lines from recurrent and primary neuroblastoma tumors which can be induced to differentiate using a variety of agents either alone or in combination. Both interferons α (IFN-α) and γ were active as differentiating agents alone and could be enhanced in activity when used in combination with either dibutyryl cyclic AMP (dbcAMP) or retinoic acid (RA). This enhancement was reflected in the induction of specific interferon-responsive genes (2-5A synthetase, and p68 kinase) at early times (6hr) during treatment. In contrast, the induction of class 1 HLA mRNAs by IFN-a was not modified in combination treatments with dbcAMP or RA. Intrinsically elevated N-myc gene expression in these cell lines was not down-regulated early in response to the differentiating agents although decreased N-myc expression was seen concomittant with neurite outgrowth. Thus these cells provide a model system for studying early molecular events in the induction-differentiation and results obtained thus far suggests a potential clinical role for combination therapy.

E 445 ANALYSIS OF IGF ACTION USING MG-63 HUMAN OSTEOSARCOMA CELLS, Richard W. Furlanetto, and Richard B. Womer, Divisions of Endocrinology/Diabetes and Oncology, The Children's Hospital of Philadelphia and Department of Pediatrics, University of Pennsylvania, Philadelphia, PA 19104 The insulin-like growth factors are important mitogens for connective tissue cells, but other growth factors (such as PDGF and EGF) are usually necesary for an optimal response to them. However, MG-63 human osteosarcoma cells which have been growth arrested in serum-free medium respond mitogenically to the IGFs in the absence of other added growth factors, and are therefore an excellent model system for studying the mechanisms by which these hormones function. In MG-63 cells IGF-I has an ED_{50} of 0.4 nM and is about 4-fold more potent that IGF-II (ED_{50} = 1.6 nM). IR-3, a monoclonal antibody to the type I IGF receptor, inhibits the mitogenic effects of both IGFs, indicating that both are operating through this receptor type. In contrast, the mitogenic effect of insulin (ED_{50} = 1.7 nM) is not inhibited by IR-3, indicating that insulin is working through its own receptor. PDGF and EGF are only weakly mitogenic for these cells and have no effect on IGF-I mitogenicity. However, TGF-β is a potent inhibitor of IGF activity, with half maximal effect at 0.2 ng/ml.

When IGF-I is added to quiescent MG-63 cells there is a lag of about 18 hours before DNA synthesis begins. This 18 hour period defines the G1 phase in these cells. Both IR-3 and antibody to IGF-I inhibit IGF-I action if added before or during the first 12 hours of G1, but at longer time intervals these antibodies loose their inhibitory effect. This indicates that IGF activity is required during the first 12 hours of G1 but that events occurring later in G1 are IGF independent.

E 446 INTERNAL DELETIONS OF THE EGF RECEPTOR GENE IN PRIMARY HUMAN GLIOMAS. Albert Wong, Sandra Bigner, Darell Bigner and Bert Vogelstein. Johns Hopkins Oncolgy Center, Baltimore, MD, 21231, and Duke University Medical Center, Durham, NC, 27710.

Alterations of the EGF receptor (EGFR) gene in primary human gliomas have been observed by us and other investigators. Our earlier work had suggested that most tumors with detectable rearrangements in the EGFR gene were due to intragenic deletions in the extracytoplasmic domain. We have characterized the structure of the rearranged EGFR genes and resulting transcripts by three different methods. We first isolated clones for the EGFR gene from a phage library. These clones were then used as hybridization probes on Southern blots made from tumor DNA. This analysis revealed that either of two different regions of the extracytoplasmic domain were deleted. Next, RNAase protection experiments with EGFR cDNA clones were used to examine mRNA from these tumors. Each type of deletion produced a characteristic transcript, even though the deletions were not identical at the genomic level. This suggested that similar exons were lost in either type of deletion. Based on data from the RNAase protection experiments, primers were chosen that flanked the deleted region. First strand cDNA was generated from tumor RNA by standard methods and followed by amplification using the polymerase chain reaction method. Sequencing of these fragments revealed that in-frame deletions occurred in the extracytoplasmic domain of the receptor in each case. Further investigations into the properties of these naturally occurring mutants may yield insights into the role of the EGF receptor in glial tumorigenesis.

E 447 **THE MOUSE LIVER SPECIFIC ENHANCER BINDING PROTEIN C/EBP IS REGULATED AT THE TRANSCRIPTIONAL LEVEL,** Kleanthis G. Xanthopoulos, Jovan Mirkovitch, and James E. Darnell, Jr., Molecular Cell Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

We have cloned the mouse DNA counterpart of the rat C/EBP, an enhancer binding protein greatly enriched in liver. The protein binds to multiple DNA sequences present in the regulatory regions of at least three mouse genes mainly expressed in liver. Sequence analysis of a λ gt-11 clone including the largest insert of mC/EBP showed a high degree of homology with the rat clone.

We have used the cDNA clone to determine: 1) the tissue distribution and the relative steady state levels of the corresponding mRNA and 2) the transcription rate of C/EBP in various mouse organs. Our results show that the mRNA is present in low levels in liver and fat tissues but not in spleen, kidney or brain, and thus it follows a tissue specific pattern of expression. Surprisingly however, the nuclear "run-on" assays demonstrated a very high transcriptional rate for this gene (approximately half that of the albumin gene) with limited tissue distribution. We therefore believe that the mRNA of mC/EBP must have a very short half life. These results demonstrate for the first time that a tissue specific enhancer binding protein is regulated at the transcriptional level.

E 448 CELLULAR GROWTH RESPONSE TO EPIDERMAL GROWTH FACTOR AND OVERPRODUCTION OF ITS RECEPTOR IN COLON CARCINOMA CELLS DERIVED FROM A GARDNER SYNDROME PATIENT, Lynn C. Yeoman, Ching-Wah Wan, Michael E. Gross, Michael G. Brattain, Diane E. Brattain, Matilde M. Olive and Bruce M. Boman, Bristol-Baylor Lab. Baylor College of Medicine, The M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030 and Creighton University Cancer Research Center, Omaha, NE 68131

The receptor binding and cellular growth response for exogenous epidermal growth factor (EGF) were studied using the DiFi colon carcinoma cell line established from the colon adenocarcinoma of a Gardner Syndrome patient. Overproduction of EGF receptors on DiFi cells was measured by Scatchard analysis of 125I-EGF binding isotherms. The number of EGF receptors per cell was calculated to be $5.70 \pm 0.08 \times 10^6$ receptors per cell. The results showed a single receptor population with an apparent dissociation constant (Kd) of 1.32 ± 0.26 nanomolar. The cellular growth response of DiFi cells to exogenous EGF was studied using a soft agarose assay. Inhibition of colony formation by EGF (50% reduction at a concentration of 0.1 nanomolar) indicated that DiFi cells express a functional EGF receptor. These results suggest a net growth inhibitory role for an overexpressed EGF receptor population in a colon carcinoma cell line derived form a Gardner syndrome patient.

E 449 CYTOKINE ACTIVITY OF ATL-DERIVED FACTOR (ADF), AN INDUCIBLE ENDOGENOUS PROTON DONOR PRODUCED BY TRANSFORMED LYMPHCYTES. Yodoi J., Tagaya Y., Yamauchi A., Inamoto T., Masutani H., Nakamura H., Maeda Y., Wakasugi H., Tursz T., Maeda M. Institute for Immunology, Faculty of Medicine, Kyoto University, Kyoto 606; Gustav Roussy Inst. Villejuif; Chest Desease Research Institute, Kyoto University, Kyoto 606

In HTLV-I-transformed T4(+) T cells established from patients with Adult T-cell Leukemia (ATL), there is an abnormal expression of IL-2R/p55(Tac). ATL-derived factor (ADF) is an IL-2R inducing soluble factor produced and released by these T cell lines. ADF cDNA was cloned from the cDNA library of HTLV-I(+) ATL-2 cell line using an oligonucleotide probe corresponding to the partial N-terminal amino acid sequence of purified ADF. ADF is a 105 amino acid peptide without 5' signal sequence, having a limited similarity to IL-1β and a significant homology to a thiol reducing co-enzyme, thioredoxin, one of the major thiol reducing co-enzymes with many biological activities.

Recombinant ADF produced by the COS-7 cells and E. coli had IL-2R/p55 inducing activity, growth promoting activity and thioredoxin-like reducing activity. Reduction of ADF was required for the optimal cytokine activity. ADF mRNA was continuously produced by HTLV-I(+) T cell lines and some EBV transformed B cell lines. ADF mRNA was also induced on normal peripheral blood lymphocytes activated by mitogen and phorbol myristate acetate. Using rabbit antibodies against C terminal 16mer ADF peptide, the production of ADF protein was demonstrated in activated lymphocytes, HTLV-I(+) T cell line cells and ATL leukemic cells in vivo. The possible relationship between reducing activity and the intra- and inter-cellular growth promoting activity of ADF is discussed.

E 450 ESTRADIOL IS SYNERGISTIC WITH IGF1 OR EGF TO STIMULATE ANCHORAGE INDEPENDENT GROWTH OF ESTROGEN RECEPTOR POSITIVE HUMAN BREAST CANCER CELLS IN SERUM SUPPLEMENTED BUT NOT IN SERUM FREE AGAR, G. Zugmaier, C. Knabbe, B. Deschauer, B.W. Ennis, M.E. Lippman and R.B. Dickson, Lombardi Cancer Research Center, Georgetown University, 3800 Reservoir Rd. Washington D.C, 20007, We tested the response of the estrogen receptor positive cell lines MCF7, (early and late passage), ZR75-1, and T47D to estradiol, IGF1, EGF and basic FGF in soft agar with or without serum (supplemented with Transferrin 2mg/l, Fibronectin 1mg/l, BSA 200mg/l). Basic FGF had no effect on these three cell lines. In serum supplemented agar the growth of early passage MCF7, ZR75-1 and T47D cells was stimulated by estradiol up to 8-fold above background. There was no effect of the tested growth factors on the growth of the 3 cell lines, except a 2-fold increase in growth induced by EGF in T47D cells. There was, however, a 1.5-fold stimulation above the estradiol effect induced by IGF1 in early passage MCF7, EGF in T47D and IGF1 or EGF in ZR75-1 cells. The spontaneously high cloning rate of late passage MCF7 cells was not further stimulated by estradiol alone or together with growth factors. In serum free anchorage independent growth IGF1 induced a 2-3 fold growth stimulation in MCF7 (early and late passage) and T47D cells, whereas EGF had this effect only in T47D cells. The two growth factors together did not induce a further stimulation. ZR75-1 cells did not clone under these conditions. The anchorage independent growth of the cell lines was not stimulated in serum free conditions by estradiol alone or in combination with IGF1 or EGF. These data indicate that the effects of estradiol and growth factors on the anchorage independent growth of estrogen receptor positive human breast cancer cell lines depend on cell type and tissue culture conditions.

Angiogenesis; FGF

E 500 KINETICS OF bFGF UPTAKE AND NUCLEAR TRANSLOCATION IN ADULT BOVINE AORTIC ENDOTHELIAL CELLS (ABAE), Véronique Baldin, Gérard Bouche, Jacqueline Depeyre, Nicole Gas, Hervé Prats and François Amalric, Centre de Recherche de Biochimie et de Génétique Cellulaires du C.N.R.S., 118 route de Narbonne, 31062 Toulouse cedex, France.

Primary cultures of ABAE cells reach up to confluence in presence of serum and bFGF. By removal of bFGF, cells growth is synchronously stopped in G1 and it can be restored by the sole addition of the growth factor. We have followed the distribution of bFGF in the exponentially growing, quiescent sparse and confluent cells by immunocytologie using a polyclonal serum raised against bFGF. In growing cells, the growth factor is detected in the cytoplasm and in the nuclei while in confluent cells, it is only barely detected into the cytoplasm. The kinetic of uptake of the growth factor was followed in quiescent sparse cells after addition of bFGF. After 2 min., most of the label is associated with the cell surface. Between 5 min. and 20 min., the growth factor is present in small vesicles, dispersed through the cytoplasm. After 20 min., most of these small vesicles collapsed and give rise to a large vesicle with perinuclear localization. After 30 min., this vesicle vanishes and most of the growth factor is recovered into the nuclei and particularly into the nucleolus. To discriminate between endogeneous bFGF and exogeneous bFGF, ^{125}I labelled bFGF was added to the culture medium. Localization of the labelled growth factor into the different cell compartments was carried out. Similar results were obtained with the presence of the growth factor inside the nucleolus and a similar kinetic of transport. A correlation was established between the appearance of bFGF inside the nucleolus and the reinitiation of rDNA transcription. Potential nuclear targets of bFGF have been characterized.

E 501 GROWTH-FACTOR DEPENDANT IN VITRO CULTURE OF KS-DERIVED CELLS, Peter Biberfeld*, Shuji Nakamura**, Zaki S. Salahuddin**, Barbara Ensoli**, Robert C. Gallo**, *Department of Pathology, Karolinska Institute, Stockholm, Sweden, **Lab. of Tumor Cell Biology, N.C.I., N.I.H., Bethesda.

Culture medium (CM) from retrovirus infected T-cell lines promote the continuous, in vitro growth of cells from biopsies of Kaposi's sarcoma (1).
These cells have endothelial like features and produce in vitro several growth factors notably bFGF-like activity (2). These cells elicit a strong angiogenic reaction in nude mice and on chicken allantoic membranes. Studies are in progress on the identity of the CM with growth promoting activity to KS-derived cells and the possible expression of autocrine and paracrine activities by these in vitro cultured cells.

1) Nakamura, S., Salahuddin, S.Z., Biberfeld, P., Ensoli, B., Markham, P.D., Larsson, L. & Gallo, R.C., Long-term culture of Kaposi's sarcoma cells by a growth factor(s) released by human retrovirus infected T4-cells (in press), Science, 1988.

2) Salahuddin, Z.S., Nakamura, S., Biberfeld, P., Kaplan, M.H., Markham, P.D., Larsson, L. & Gallo, R.C., Cultured Kaposi's sarcoma cells characterization and induction of KS-like lesions (in press)., Science, 1988.

E 502 EXPRESSION OF ACIDIC FIBROBLAST GROWTH FACTOR IN NIH/3T3 CELLS WITH AND WITHOUT THE ADDITION OF A SECRETION SIGNAL SEQUENCE, Shelley B. Blam[1], Edmund Tischer[2], Judith A. Abraham[2], and Stuart A. Aaronson[1], 1) LCMB, NCI, NIH, Bldg. 37, Rm. 1E24, Bethesda, Maryland 20892, 2) California Biotechnology, Inc., 2450 Bayshore Pkwy., Mountain View, CA 94043

To examine the oncogenic potential of acidic fibroblast growth factor (aFGF), which appears to lack a classical secretion signal peptide, the human aFGF coding region was expressed in NIH/3T3 cells under the control of either the long terminal repeat of Moloney murine leukemia virus or the human metallothionein-II_A promoter. The gene produced a low but reproducible number of transformed foci with an efficiency of 4-8 ffu/μg. In contrast, introduction of the aFGF coding sequence linked to the human growth hormone signal peptide sequence produced rapid morphological changes in NIH/3T3 cells with an increased efficiency of $3x10^4$ ffu/μg. Both the wild-type and chimeric genes produced cell-associated aFGF that were identical in size. Transformants induced by either aFGF or the signal-peptide chimera formed colonies in soft agar and induced tumors in nude mice. These results indicate that transfection of NIH/3T3 cells with aFGF cDNA results in malignant cell transformation at a low frequency and this effect is greatly enhanced by the addition of a secretion signal sequence.

E 503 CLONING AND EXPRESSION OF cDNA AND GENOMIC DNA CODING FOR HUMAN HEPARIN-BINDING GROWTH FACTOR-1, Ing-Ming Chiu, Wen-Pin Wang, Phanpimol Bunnag, Kirsten Lehtoma and Mariet Lee Varban, Department of Internal Medicine and Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210.

Endothelial cell polypeptide mitogens have been described and are generically designated as heparin-binding growth factors (HBGF). A comparison of the protein sequences and biological properties has shown that all HBGF described so far, belong to either class 1 or class 2 HBGF. These growth factors are important to the maintenance and regulation of blood vessel homeostasis and may be important in the pathobiology of tumor growth. Recently, three oncogenes, mouse int-2, human hst/KS3 and FGF-5, have been shown to be homologous to HBGF. We have identified several overlapping cDNA clones coding for human class 1 HBGF (HBGF-1) from a human brain stem cDNA library. We have also isolated overlapping genomic DNA clones by screening human genomic DNA libraries using HBGF-1 cDNA probes. The exon-intron structure of the HBGF-1 gene was determined by nucleotide sequence analysis. Southern blot analysis of human DNA suggested that there is a single copy gene coding for HBGF-1. A 4.5 kb mRNA homologous to the HBGF-1 cDNA was detected in RNA isolated from human brain, kidney and several human fetal tissues. We have also constructed retroviral expression vectors containing the full length coding sequence of HBGF-1. These constructs were introduced into NIH/3T3 cells by cotransfection with pSV2neo. G418-resistant cell lines were shown to express the HBGF-1 mRNA and the protein. These cells were conferred some properties of the transformed phenotype distinct from those of the perental NIH/3T3 cells. With the availability of these HBGF-1 producing cell lines, it is now possible to address the question of the roles of HBGF-1 in tumor formation either directly through an autocrine growth factor activation mechanism or indirectly through angiogenesis.

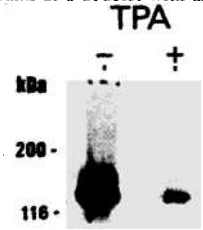
E 504 INTERLEUKIN-1 INHIBITORS PROMOTE ENDOTHELIAL CELL GROWTH IN VITRO Federico Cozzolino, Maria Torcia and Donatella Aldinucci, IV Department of Internal Medicine, University of Firenze, Viale Pieraccini 18, I-50139 Firenze, Italy.

Interleukin-1 (IL-1) exerts several biologic activities on Endothelial Cells, such as induction of Procoagulant Activity (PCA) expression and prostaglandin release. Recently, it has been observed that IL-1 potently modulates Endothelial Cell proliferation in vitro in a dose-dependent fashion, following interaction with a specific surface receptor. We have investigated the mechanisms whereby suppressor cells from metastatic nodes or tumor-infiltrating lymphocytes inhibit the immune response against cancer cells. We could observe that culture supernatants of suppressor cells contained a strong IL-1-inhibitory activity, both in the LAP and in the PCA assays. Gel fractionation of supernatants revealed a protein with about 65 kDa MW. When tested on Endothelial Cell proliferation assays, it could completely revert the IL-1 effect on cell growth. In addition, it completely blocked the IL-1-induced PCA generation. We suggest that production of this protein by cells infiltrating the tumor site may both enhance angiogenesis and preserve patency of tumor vasculature.

E 505 THE HUMAN aFGF GENE ENCODES MULTIPLE FUNCTIONAL POLYADENYLATION SITES. Gregg R. Crumley, Richard D. Howk, Mark W. Ravera and Michael Jaye, Rorer Biotechnology, Inc., 680 Allendale Rd., King of Prussia, PA 19406. We previously reported the isolation of two cDNA clones for human acidic fibroblast growth factor (aFGF). The nucleotide sequence through the coding region and the deduced amino acid sequence were presented. The previous cDNA clones accounted for 2.2Kb of a human brain messenger RNA estimated to be greater than 4Kb in length. We have used the original cDNA and a 3'-specific 30-base oligonucleotide to isolate a new set of overlapping cDNA clones. The new clones reveal four alternative 3' cleavage and polyadenylation sites. The most abundant clones terminate at the most distal site 3,100 bases downstream of the coding region. The composite nucleotide sequence of the overlapping clones accounts for 3,618 bases of the aFGF mRNA. Multiple regulatory elements are located within the long 3' non-translated region, including the classical AATAAA hexamer upstream of the cleavage sites and a 12bp element located downstream of the cleavage sites. Additional potential polyadenylation signals are found elsewhere in the 3' region and six copies of the ATTTA destabilization motif are detected. Northern blot analysis reveals three mRNA species whose sizes and intensities correlate with the location of alternative polyadenylation sites and the relative abundance of cDNA clones representing them.

E 506 ISOLATION AND CHARACTERIZATION OF FIBROBLAST GROWTH FACTOR GENES. O. de Lapeyrière, I. Marics, J. Adélaïde, F. Raybaud, F. Coulier, O. Rosnet, D. Benharroch, M.G. Mattei[1] and D. Birnbaum, U.119 INSERM, 27, Bd Leï Roure, 13009 Marseille, [1]U.242, Hôpital de la Timone, 13005 Marseille. Fibroblast growth factor (FGF) genes are encoding known (acidic and basic FGF) and putative growth factors (HST, INT.2 and FGF.5) involved in various fundamental processes such as mitogenesis, angiogenesis, morphogenesis and possibly also carcinogenesis. The number of members of the FGF family remains unknown. By screening genomic libraries with a HST probe in reduced conditions of stringency, we have isolated a new member of the family, the FGF.6 gene. This gene presents strong sequence homology with HST. The gene seems well conserved through species. Unlike HST, FGF.6 has never been identified in NIH3T3 assays. However, like HST, the cloned normal human FGF.6 gene is able to transform NIH 3T3 cells. So far FGF.6 has not been implicated in any tumor tested. FGF.6 is not linked to the tandem HST/INT.2 on chromosomal band 11q13 but located on 12p13. Expression of FGF-6 is being studied on mouse tissues. Focus-assays in low serum concentration allowed us to identify two other FGF-related sequences in NIH 3T3 transformants, hybridizing respectively to HST and INT.2 probes in reduced stringency conditions.

E 507 **PROTEIN KINASE C-MEDIATED MODULATION OF THE BASIC FGF RECEPTOR IN CAPILLARY ENDOTHELIAL CELLS, Susan R. Doctrow, Harvard Medical School and Childrens Hospital, Boston, MA 02115.** Angiogenesis involves not only increased proliferation of capillary endothelial cells but also organization of these cells into non-proliferating, differentiated blood vessels. The intracellular events regulating endothelial cell proliferation in response to angiogenic mitogens are not known. We have previously shown that, upon treatment with activators of protein kinase C (PKC), bovine adrenal capillary endothelial (BCE) cells do not proliferate or synthesize DNA in response to the potent mitogen basic FGF (bFGF) (Doctrow and Folkman, J. Cell Biol. 104:679). The present study suggests that PKC activators may "desensitize" BCE cells to bFGF through modulation of the bFGF receptor. A high affinity receptor has been identified by crosslinking of ^{125}I-bFGF to BCE cells. The receptor-^{125}I-bFGF complex appears on SDS-PAGE autoradiograms as a doublet with an apparent molecular weight of 130/150 kDa. In BCE cells treated with the PKC activator tetradecanoylphorbol acetate (TPA), the amount of receptor detected by subsequent crosslinking is dramatically reduced (see autoradiograph). The concentrations of TPA required for this effect agree with those that activate PKC in intact cells (K_{50} = 10 nM). Control experiments show that TPA does not decrease recovery of cellular protein or increase ^{125}I-bFGF degradation. TPA-induced reduction of detectable bFGF receptor occurs with a $t_{1/2}$ (at 37°C) of about 30 min, and thus is the earliest reported action of TPA on these cells. (By comparison, the characteristic "sprouting" morphology exhibited by TPA-treated BCE cells is not visible until 60-120 min.) These findings support a PKC-mediated decrease in bFGF receptor number in BCE cells. Whether PKC causes down regulation, perhaps mediated by direct phosphorylation, of the receptor remains to be determined.



E 508 THE DISTRIBUTION OF BASIC FGF mRNA IN RAT BRAIN, Naoya Emoto, Patricia Walicke*, Ana Maria Gonzalez, Shunichi Shimasaki, and Andrew Baird, Laboratories for Neuroendocrinology, The Salk Institute, 10010 N. Torrey Pines Road, La Jolla, CA 92037, *Department of Neuroscience, University of California, San Diego, CA 92093

The brain, unlike almost all other tissues, is characterized by the fact that it contains significant quantities of mRNA transcripts encoding basic fibroblast growth factor (FGF). Because this observation suggests that the regulation of basic FGF synthesis is different in the central nervous system, we have sought to compare the amounts of mitogen with the mRNA encoding the growth factor.

The distribution of FGF mRNA in the rat brain has been studied using a cDNA encoding rat basic FGF. A single hybridizing mRNA of 6.0 Kb in length was clearly detected in the total RNA (10 μg) prepared from hypothalamus, frontal cortex, parietal cortex, occipital cortex, hippocampus and pons. In contrast, in the total RNA (30 μg) prepared from the cerebellum and pituitary, a similar hybridizing band of 6.0 Kb was barely detectable. In each of these tissues, however, immunohistochemical staining of sections, using a polyclonal antisera to a fragment of bovine basic FGF or a monoclonal antisera to recombinant human basic FGF, revealed the presence of the growth factor. In an effort to establish the cell type responsible for the synthesis of basic FGF, neurons were prepared from 18-day fetal rat telencephalon and astrocytes were prepared from the neonatal rat brain. Although Northern analyses of the total (15 μg) and poly A^+ selected (5 μg) mRNA prepared from neurons failed to detect basic FGF, a 6.0-Kb transcript, indistinguishable from the one observed in brain, was detectable in the mRNA prepared from astrocytes. The results suggest that astrocytes and not neurons are responsible for the elevated mRNA detected in brain. They also support the concept that astrocytes produce the neuronotrophic factors that are required to ensure neuronal survival and differentiated function. Because neurons can be shown to possess a specific receptor for basic FGF, the results are strongly suggestive that, in the brain, this mitogen plays the function of a neuronotrophic rather than angiogenic factor.

E 509 **COMPONENTS OF THE EXTRACELLULAR MATRIX DIFFERENTIALLY REGULATE THE PHOSPHORYLATION OF BASIC FGF**, Jean-Jacques Feige and Andrew Baird, Laboratories for Neuroendocrinology, The Salk Institute, La Jolla, CA 92037, USA.

Basic FGF (bFGF) is a substrate for protein kinase C (PKC) and cyclic AMP-dependent protein kinase (PKA) and is phosphorylated by endothelial cells and hepatoma cells in culture. Components of the extracellular matrix (ECM) can differentially regulate the phosphorylation of basic FGF. Glycosaminoglycans inhibit PKC-mediated bFGF phosphorylation whereas fibronectin, laminin and collagen IV have no effect.
In contrast, these ECM-derived proteins inhibit and heparin stimulates PKA-mediated bFGF phosphorylation. The enhancement of PKA-mediated bFGF phosphorylation observed in the presence of heparin results from an increased Vmax of the reaction (with no modification of the Km) and a shift of the phosphorylation site from thr112 to ser64. These observations suggest that the interactions between bFGF and components of the ECM can result in enough conformational change to regulate post-translational modifications of FGF. This offers a new mechanism for the ECM to regulate the biological activity and bioavailability of bFGF and identifies a novel interaction between FGF and fibronectin laminin and collagen.

E 510 THE HUMAN bFGF GENE ENCODES FOUR POLYPEPTIDES: THREE INITIATE TRANSLATION FROM NON-AUG CODONS. Robert Z. Florkiewicz, The Whittier Institute, 9894 Genesee Avenue, La Jolla, California 92037; and Andreas Sommer, Synergen Inc., Boulder, Colorado 80301. Sequence analysis of cDNA and genomic clones of the human basic fibroblast growth factor gene predicts a single 18kD (155 amino acid) protein. However, we show that the human hepatoma cell line SK HEP-1 co-expresses three higher molecular weight (24, 23, and 22kD) species of bFGF in addition to the 18kD protein. Using a cDNA clone derived from SK HEP-1 cells we show, by <u>in vitro</u> transcription/translation and <u>in vivo</u> COS-1 cell expression coupled with selective mutagenesis of this cDNA clone, that the 18kD protein is translationally initiated at an AUG codon while the 22, 23 and 24kD proteins are translationally initiated at CUG codons.

E 511 PITUITARY FGFs: CHARACTERIZATION OF ACIDIC AND BASIC FORMS, Angelo G. Gambarini*, Paulo L. Ho*, Ross Jakes+ and Fredrick D. Nortrop+, Department of Biochemistry*, University of São Paulo, SP 01498, Brazil and MRC Laboratory of Molecular Biology+, Cambridge CB2 2QH, UK.

Extraction of bovine pituitaries at pH 7.0, in the presence or absence of protease inhibitors, yielded both acidic and basic FGFs that were separated by heparin-affinity chromatography prior to characterization by Western blotting and N-terminal sequence analysis. Acidic FGF showed several active components of pI 4.5-6.5. The most active component, that eluted form the heparin-Sepharose column with 1.2 M NaCl, reacted with and was neutralized by an anti-brain acidic FGF and had a pI of 5 and 17 kDa molecular weight. These results suggest a close relationship between the pituitary acidic FGF and the truncated brain acidic FGF [7-140]. Basic FGF comprised: an 18 kDa form that is similar, if not identical, to the basic FGF [1-146] already sequenced by others; a 17 kDa form that seems to be a new truncated molecular species [11-146] and a set of components of about 29 kDa. The latter eluted from the heparin-Sepharose column with 1.6-1.8 M NaCl and was recognized by anti-pituitary basic FGF antiserum. These results suggest that the 29 kDa basic component might be related to the FGF's family.

E 512 A DISTINCT FACTOR IN RAT OVARIAN GRANULOSA CELL-CONDITIONED MEDIUM (GCCM) THAT STIMULATES ENDOTHELIAL CELL (EC) MIGRATION AND ANGIOGENESIS IN VITRO, Robert D. Koos, Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland 21201

The granulosa cells, which line the central avascular compartment of the ovarian follicle and developing corpus luteum, are a probable source of angiogenic factors (Koos RD, Endocrinology 119:481-489, 1986). One high molecular weight factor in GCCM causes marked changes in EC morphology and behavior; interestingly, its level in GCCM is enhanced when the granulosa cells are cultured under hypoxic conditions. In the present study, the effect of this factor on ECs grown on a collagen substrate was examined. Fetal bovine aortic ECs were grown to confluence on a thick, type I collagen gel. GCCM was ultrafiltered through a 30 kDa-cutoff membrane and the retentate added to the ECs. Within 48 h, many ECs had elongated and reorganized to form a complex network of capillary-like cords under the original monolayer; branchs of the cords could be seen deep within the collagen gel. Many of the multicellular branches appeared to contain central lumens. This was confirmed by electron microscopy. The factor that induces these changes is heat stable but destroyed by trypsin or acid treatment. It is retained by a 50 kDa, but not a 100 kDa, ultrafiltration membrane and is precipitated by 60% ammonium sulfate. The factor does not bind to heparin. The ability of this in vitro angiogenic factor (IVAF) to promote EC migration and differentiation suggests that it may play an important role in the angiogenic process. Supported by NIH CA45055.

E 513 Purification of Basic Fibroblast Growth Factor Receptor From Chicken Embryos. Pauline Lee, Victor A. Fried and Lewis T. Williams, Howard Hughes Medical Institute, Univ. of California, San Francisco and St. Jude Children's Hospital, Memphis, Tennessee 38101

Fibroblasts growth factors (FGF's) are a family of polypeptide growth factors which are potent mitogens for cells of mesenchymal origin and neural cells. In particular, FGF is mitogenic for endothelial cells and therefore plays an important role in angiogenesis and wound healing. The presence of FGF in embryonic tissues also suggests a role of this family of mitogens in development. The mitogenic effect of FGF requires interaction with high affinity cell surface receptors. We have purified a receptor for basic fibroblast growth factor (bFGFr) from white leghorn chicken embryos. Chicken embryos contain high levels of bFGFr as determined by membrane binding and cross-linking of ^{125}I-FGF. The receptor was partially purified by lectin chromatography using wheat germ agglutinin Sepharose 4B. bFGFr was further purified by ligand affinity chromatography using biotinylated human basic FGF. Ligand affinity chromatography was successful because biotin-FGF was identical to unmodified FGF as assessed by (a) competition for binding of ^{125}I-FGF to Swiss 3T3 cells, (b) direct binding to cells and (c) in vivo stimulation of tyrosine phosphorylation of a 90 kDa substrate protein. Using this purification procedure, we were able to purify 100µg of FGF receptor (1-5ng/embryo).

E 514 AMPLIFICATION OF INT-2 IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK, W. Davis Merritt, Mark C. Weissler and Tona M. Gilmer*, Department of Otolaryngology, University of North Carolina, Chapel Hill, NC 27514, and *Department of Chemotherapy, Glaxo, Inc., Research Triangle Park, NC 27709.

Int-2 is a cellular oncogene first described as a viral integration site in MMTV induced mouse mammary carcinomas. In these tumors int-2 is abnormally expressed as a result of MMTV integration. To date, no transcripts have been detected in normal adult tissues. Int-2 shares amino acid sequence similarity with a family of genes related to acidic and basic fibroblast growth factors (FGFs), two proteins with angiogenic activity. We examined 21 freshly frozen human squamous cell carcinomas for amplification of ten cellular oncogenes. Six of 21 tumors examined showed a two to ten-fold amplification of the int-2 gene. Two of these six patients also demonstrated int-2 amplification in cervical lymph node metastases removed with their tumors. No amplifications were found for eight of the other genes examined: *l*-myc, n-myc, *h*-ras, n-ras, k-ras, erb-B, neu and raf. Two patients demonstrated amplification of the c-myc gene. Because of the wide variety of mitogenic functions of acidic and basic FGFs, it is intriguing to speculate a role for expression of int-2 in the initiation or progression of squamous cell carcinoma of the head and neck. We are currently examining these tumors for expression of the int-2 RNA transcript.

E 515 ANGIOGENIC PROPERTIES OF KAPOSI'S SARCOMA DERIVED CELLS AFTER LONG-TERM CULTURE IN VITRO, Shuji Nakamura[1], S. Zaki Salahuddin[1], Shinsaku Sakurada[1], Peter Biberfeld[2], Mark H. Kaplan[3], and Robert C. Gallo[1], [1]National Cancer Institute, Bethesda, MD, [2]Karolinska Institute, Sweden, [3]North Shore University Hospital, Cornell Medical School, NY

Cells were established from Kaposi's sarcoma lesions in lung and pleural fluids from AIDS patients with Kaposi's sarcoma (KS) with the aid of a recently described growth factor(s). These long-term cultured AIDS-KS cells were shown by morphological, biological, immunological, and cytochemical analyses to share some of the phenotypic expression of endothelial cells. Further studies showed that these cells produce factors with diverse biological activities including the ability to support their own growth (autocrine) and that of normal endothelial and stromal cells (paracrine). They also contained potent angiogenic activity, as detected by chrioallantoic membrane and nude mouse assays. When the cultured AIDS-KS cells were inoculated into nude mice, they also induced the development of lesions composed entirely of murine cells, with histological features similar to that of man. This system, therefore, provides potentially important <u>in vitro</u> and <u>in vivo</u> models for the study of the pathogenesis of AIDS-KS. We suggest from these and other results that AIDS-associated and perhaps other types of KS may develop in discrete stages with the eventual formation of the AIDS-KS cells. These cells, in turn, release factors which support their own growth and the accumulation and growth of other cells which form the developing AIDS-KS lesion in a multifocal fashion.

E 116 **FGF UTILIZES A NOVEL SIGNAL TRANSDUCTION PATHWAY IN SWISS 3T3 FIBROBLASTS,** **Eeva Nånberg, Clive Morris, Francisco Vara, Theresa Higgins and Enrique Rozengurt, Imperial Cancer Research Fund, P O Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.**

The family of fibroblast growth factors is increasingly implicated in cell growth, angiogenesis, differentiation and oncogenesis. In spite of its potential importance, little is known about the signal transduction pathways activated by this growth factor. We now demonstrate that recombinant basic FGF utilizes a novel signal transduction pathway which can be distinguished from that activated by bombesin or PDGF. bFGF at mitogenic concentrations, activated PKC, measured as 80K phosphorylation. This effect was rapid reaching maximum at 10 min (EC_{50} 0.08 nM). In parallel experiments, bFGF had no effect on inositide breakdown or Ca^{2+} mobilization whereas bombesin and PDGF had an effect on both events in the presence of PKC activation. PKC-dependent events such as transmodulation of the EGF receptor, cytoplasmic alkalinization and enhancement of cAMP production, were also stimulated by bFGF. Down-regulation of PKC by prolonged pretreatment with phorbol esters prevented 80K phosphorylation and other PKC-dependent events induced by bombesin or PBt_2. The effect of bFGF on 80K phosphorylation was abolished in PKC down-regulated cells while EGF receptor transmodulation and alkalization were partially inhibited. These data indicate that bFGF activated PKC in the absence of phosphoinositide turnover and Ca^{2+} mobilization. In addition, bFGF can activate the cells via a PKC independent pathway(s). The findings support the hypothesis that mitogenesis is stimulated by multiple signal transduction pathways.

E 517 ISOLATION AND CHARACTERIZATION OF THE PROTEIN ENCODED BY THE K-fgf ONCOGENE, Karen M. Newman, Anna Maria Curatola and Claudio Basilico, Department of Pathology, New York University Medical Center, New York, NY 10016.
The protein encoded by the K-fgf/hst oncogene is a growth factor (K-FGF) belonging to the FGF family. Similar to other members of this family, K-FGF interacts with heparin which enhances its stability after secretion into culture medium. We constructed a CHO cell line expressing and secreting a high level of K-FGF and we used its conditioned medium to isolate the protein by heparin affinity chromatography. The protein elutes from this column at a salt concentration similar to acidic FGF. The elution pattern of the K-FGF was determined by the ability to induce morphological changes in NIH3T3 cells and to stimulate DNA synthesis in serum-arrested Balb3T3 cells as well as by immunoblotting using a rabbit antiserum raised against a K-FGF bacterial fusion protein. The properties of the protein are currently under investigation and will be discussed.

E 518 A NEW LUNG-DERIVED GROWTH FACTOR (LDGF-1) DIFFERENTIALLY STIMULATES THE GROWTH OF TUMOR CELLS METASTATIC TO LUNG. Garth L. Nicolson and Philip G. Cavanaugh, Dept. of Tumor Biology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030.

The ability of malignant cells to respond to growth factor(s) present in or secreted by a target organ may play an important role in tumor metastasis. We used metastatic cell lines and clones of the rat 13762NF mammary adenocarcinoma. Whereas poorly lung-metastatic MTPa and MTC cells did not grow in response to lung-conditioned medium, highly lung-metastatic MTLn3 cells responded and grew rapidly in lung-conditioned medium. The major growth-promoting factor for MTLn3 cells from porcine and rat lung-conditioned media was purified using hydroxylapatite and anion exchange chromatgraphy, chromatofocusing, size exclusion chromatography, and native gel electrophoresis. The activity in each of the purification fractions was measured by determining the ability to increase the number of MTLn3 cells in serum-deprived culture medium. The responsible component for differentially stimulating the growth of highly metastatic MTLn3 cells was a glycoprotein of Mr ~66,000. Under reducing conditions, its apparent Mr was ~72,000. This lung-derived mitogen was stable at pH's of 4.0 - 9.0, had a pI of 6.9 - 7.0, and preferentially promoted the growth of lung-metastasizing tumor lines over their poorly lung-metastasizing counterparts in three tumor systems: rat 13762NF mammary adenocarcinoma, murine B16 melanoma, and murine RAW117 large cell lymphoma. The activity of LDGF-1 was not affected by pretreatment with antibodies to insulin, GM-CSF, human growth hormone, PDGF, or EGF. The results suggest that specific, possibly novel, organ growth factors are important in metastatic colonization and organ growth of particular malignant cells.

Supported by NCI grant R35-CA44352 (OIG) to G. L. Nicolson

E 519 TRANSFORMING GROWTH FACTOR ß (TGF-ß) INDUCTION OF ENDOTHELIAL CELL (EC) ORNITHINE DECARBOXYLASE (ODC), Urszula Orlinska, Bernhard Hennig, Mark Gillespie, and Jack W. Olson, University of Kentucky, College of Pharmacy, Lexington, KY 40536.
TGF-ß is angiogenic in vivo and can elicit an angiogenic like response in vitro. Since angiogenesis is a complex process which involves the synthesis of proteins, RNA and DNA by EC's, we hypothesized that TGF-ß would increase EC synthesis of polyamines. We have shown previously that the activity of ODC, the generally rate limiting enzyme in polyamine biosynthesis, was increased in cultured porcine pulmonary artery EC's in a concentration dependent fashion by TGF-ß (0.05 to 5 ng/ml). Our present studies demonstrate that 5 ng/ml of TGF-ß maximally stimulated EC ODC activity after a 12 hour incubation and significantly elevated EC content of the polyamines putrescine, spermidine and spermine. To evaluate possible transmembrane signaling pathways involved in TGF-ß activation of EC ODC we examined the effect of a 3 hour pretreatment with varying concentrations of pertussis toxin. Pertussis toxin significantly inhibited TGF-ß stimulated ODC activity and induced ADP-ribosylation in a concentration dependent fashion (0.6 to 80 ng/ml). Further evidence for possible involvement of G proteins was provided by the ability of TGF-ß to significantly stimulate GTPase activity in isolated EC membranes. To assess the role of protein kinase C, the effects of the protein kinase C inhibitors H-7, H-9, and staurosporine were evaluated. All compounds inhibited TGF-ß-induced ODC activity in a concentration-related manner with IC-50 values of 2.6 and 5 µM for H-7 & H-9 respectively, and 1.4 nM for staurosporine. As determined by immunopreciptation studies both PT and H-7 significantly reduced the amount of ODC protein in TGF-ß treated EC's. These observations suggest that TGF-ß induction of ODC in EC's utilizes both G-protein and protein kinase C signaling pathways. (Supported by HL-36404, HL-38495, and AHA-KY).

E 520 HIGH LEVEL OF EXPRESSION OF BASIC FGF IS REQUIRED TO INDUCE THE TRANSFORMED PHENOTYPE, N. Quarto[1], D. Talarico[2], D. Moscatelli[1], R. Florkowicz[3], C. Basilico[2] and D. B. Rifkin[1], [1]Dept. of Cell Biology, Kaplan Cancer Center New York University School of Medicine, The Raymond and Beverly Sackler Foundation, [2]Dept. of Pathology, 550 First Aven., New York, New York 10016 and [3]Synergen, Inc. Boulder, CO.
Three new oncogenes encoding proteins homologous to FGF have been recently isolated, suggesting that FGF might be a potential transforming gene. In order to test this hypothesis, human bFGF cDNA has been inserted into a retroviral vector and introduced into NIH 3T3 cells either by transfection or by infection. Our results show that most transfected cells acquired the transformed phenotype, and were able to grow in soft agar and to induce tumors in nude mice, while infected cells did not. Western blot analysis revealed that the transformed phenotype was related to the expression of very high levels of bFGF. Furthermore, in cells producing high amounts of bFGF, down regulation of bFGF receptors occurred.

E 521 INTRODUCTION OF A "TRANSFORMING" GENE (EJras OR SV40-LT) INTO NORMAL, DIPLOID HUMAN MESOTHELIAL CELLS, FIBROBLASTS, AND EPIDERMAL KERATINOCYTES INDUCES EGF-INDEPENDENCE AND A PRENEOPLASTIC STATE ASSOCIATED WITH PRODUCTION OF AN AUTOCRINE, HEPARIN-BINDING GROWTH FACTOR. James Rheinwald, George Cicila, Denis Henrard, Alan Thornley, Ross Tubo, Beatrice Zenzie, and Therese O'Connell, Dana-Farber Cancer Institute and Department of Cellular and Molecular Physiology, Harvard Medical School, Boston, MA 02115
Earlier work from this laboratory identified the mitogen requirements for rapid (T_d~24 hrs) and long-term (≥45 doublings before senescence) growth of normal human mesothelial cells (the simple squamous epithelial cell that covers the surfaces lining the pleural, peritoneal, and pericardial cavities) and keratinocytes (the cell that forms stratified squamous epithelia such as the epidermis) in culture. These cell types slow their grow markedly and can become growth arrested when they are deprived of EGF but their "naturally" transformed counterparts, mesothelioma and squamous cell carcinoma (SCC), grow independently of EGF. Introduction by $CaPO_4$ transfection or defective retroviral transduction of either the mutant c-Hras gene EJras or the SV40 large T antigen (SVLT) gene into these cells confers the ability to grow independently of EGF but does not generally lead to complete, malignant transformation. Xenografting experiments on nude mice indicate that EJras-transduced keratinocytes form a severely dysplastic epithelium. Conditioned medium from EJras- and SVLT-transfectants and from mesothelioma and SCC cell lines contain a factor that can replace EGF as a mitogen for normal mesothelial cells. We have found that this factor is not TGF-α or EGF, but instead is a heparin-binding growth factor which we are now characterizing.

E 522 MOLECULAR CLONING OF RAT OVARIAN BASIC FGF cDNA AND TISSUE DISTRIBUTION STUDY OF ITS mRNA, Shunichi Shimasaki, Naoya Emoto, Futoshi Shibata, Andrew Baird, and Nicholas Ling, Laboratories for Neuroendocrinology, The Salk Institute, 10010 N. Torrey Pines Road, La Jolla, CA 92037

Basic fibroblast growth factor (FGF) is a potent mitogen for vascular and capillary endothelial cells as well as many cells of mesodermal or neuroectodermal origin. To study the regulation of basic FGF synthesis and secretion, we have isolated three cDNAs encoding rat basic FGF from 10^6 independent clones prepared from a pregnant mare's serum gonadotropin-stimulated ovarian cDNA library. One of the cDNAs contained the entire coding sequence for basic FGF. The predicted sequence of rat basic FGF is one amino acid shorter than the human and bovine sequences and contains 5 conservative amino acid substitutions. Using this probe, we have studied the distribution of basic FGF mRNA in tissues.

Poly A^+ RNA from brain cortex and hypothalamus showed a single 6.0-Kb band by Northern analyses, but basic FGF mRNA was undetectable in adrenal, spleen, heart, lung, kidney, liver, stomach, small intestine, large intestine, testis, and ovary. In contrast, mouse macrophages can be shown to express at 6.0 Kb transcript that hybridizes with the rat FGF cDNA. Furthermore, incubation of these cells with either transforming growth factor-β or endotoxin increases the amount of FGF mRNA detected. The results confirm the capacity of stimulated mouse macrophages to express basic FGF and support the notion that the high levels of the protein found in tissues are due to storage of the mitogen in the extracellular matrix and not to elevated gene expression. The significance of the high abundance of mRNA in tissues such as brain cortex and hypothalamus which are not undergoing either active angiogenesis or cell proliferation is unclear, but emphasizes the potential neuronotrophic function of basic FGF *in vivo*.

E 523 TWO mRNA'S ARE HOMOLOGOUS TO THE GENE FOR ACIDIC FIBROBLAST GROWTH FACTOR. Deborah Sullivan and Thomas Storch, Pediatrics Department, Tulane University Medical Center, New Orleans, LA 70112
Tumor growth in vivo depends on continued expansion of the vascular system that supplies the tumor with O_2 and nutrients. Acidic fibroblast growth factor (aFGF), which contributes to blood vessel formation by inducing endothelial cell migration and multiplication, is synthesized by a number of tumor lines. The expression of the gene that encodes aFGF was studied in cultures of medullary thyroid carcinoma cells (rat, line 6-23). To approximate the O_2 atmosphere of cells in vivo, culture conditions of 2.5% O_2, rather than 20% ambient O_2, were used. mRNA was extracted from SDS/proteinase K cell lysates with oligo-dT; residual DNA was digested with DNAase; mRNA was size-separated on 1.5% agarose/formaldehyde gels, and transferred to nylon membranes; an RNA ladder was included as a mw standard. A 485 bp cDNA to the full amino acid sequence of human aFGF was used to measure the number and length of aFGF mRNA's; only one aFGF mRNA of 4.6 kb had been found with this probe. We identified two mRNA's that hybridize to this probe under stringent conditions (0.2xSSC/62^{0}C washes): 1) a 1.4kb mRNA not previously reported; and 2) a 4.6 kb mRNA. The 1.4 kb and 4.4 kb mRNA's were both associated with polyribosomes (prepared by sucrose gradient centrifugation of NP-40 cell extracts). To compare the origin, formation and translation products of the 1.4, and 4.4 aFGF mRNA's, cDNA's have been synthesized. Nucleotide sequencing and in vitro translation are in progress.

E 524 FIBROBLAST GROWTH FACTOR (FGF) INDUCES TRANSIENT INCREASES IN FREE INTRACELLULAR CALCIUM (CA_i) THAT ARE UNRELATED TO MITOGENESIS, Robert W. Tucker, Danielle Ferris, Carmen C. Cardona, and David T. Chang, Johns Hopkins Oncology Center, Baltimore, MD 21205.
FGF is both a potent mitogen and angiogenic factor, but the intracellular signals responsible for these diverse physiological effects have not been defined. In order to determine whether increases in Ca_i are important in mitogenesis, we measured spatial/temporal changes in Ca_i in single fibroblasts (BALB/c 3T3 cells) stimulated with varying concentrations of FGF. In contrast to published reports, we found unequivocal evidence that FGF stimulates Ca_i increases that are independent of extracellular Ca^{2+}, presumably due to release ofintracellular Ca^{2+} stores. Moreover, unlike PDGF, FGF did not induce Ca_i increases that correlated with mitogenic potency. High concentrations of both recombinant acidic FGF and purified basic FGF produced maximal mitogenesis, but failed to induce Ca_i increases. Low concentrations of FGF did induce Ca_i increases, but with very little accompanying mitogenesis. Finally, loading with Quin 2/AM prevented the induction of Ca_i transients by FGF, but did not prevent the induction of DNA synthesis. Thus, FGF induces a transient release of intracellular calcium and accompanying increases of Ca_i that are not required for mitogenesis. Such signals may instead be required for the angiogenic effect of FGF.

E 525 TRANSFORMING GROWTH FACTOR TYPE-ß IS A POTENT ANGIOGENIC FACTOR, E.Y. Yang and H.L. Moses, Dept. of Cell Biology, Vanderbilt University, Nashville, TN 37232
Transforming growth factor type-ß (TGFß) is thought to play a major role in wound healing. It is released from platelets during degranulation, and its injection subcutaneously into new born mice causes the formation of granulation tissue. It is paradoxical, however, that TGFß stimulates granulation tissue capillary formation in vivo, and yet in vitro TGFß inhibits endothelial cell proliferation. To examine this paradox more carefully we have studied the angiogenic effects of TGFß in a more traditional angiogenesis assay, the chicken chorioallantoic membrane (CAM). Application of TGFß to the CAM for 3 days between days 9 and 12 post-fertilization was found to potently stimulate angiogenesis. TGFß induced angiogenesis was as efficacious as that stimulated by basic fibroblast growth factor and dose responsive between 10 and 100 ng of TGFß. Histologic examination at 3 days TGFß treatment revealed increased numbers of arterioles and capillaries, ectodermal and endodermal epithelial proliferation, and abundant fibroblastic proliferation within the mesenchyme. Numerous inflammatory cells were also evident at this time. After 24 hours TGFß treatment, however, increased numbers of capillaries were visible at the ectodermal/mesenchymal interface without detection of inflammatory cells. These results suggest that TGFß acts either directly or indirectly to stimulate angiogenesis in the CAM assay. Direct TGFß action in stimulating endothelial cell growth may only be seen in vivo where the extracellular matrix or hormonal microenvironment context is permissive. Indirect TGFß angiogenic activity may occur through stimulating angiogenic factor production by a non-inflammatory intermediary cell type.