GENETIC MECHANISMS IN CARCINOGENESIS AND TUMOR PROGRESSION

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Plenary Sessions Page

Poster Sessions

Genetic Mechanisms in Carcinogenesis and Tumor Progression

Genomic Stability-I

D 001 ROLE OF GENETIC INSTABILITY IN CARCINOGENESIS, J. Carl Barrett, Takeki Tsutsui and Mitsuo Oshimura, Laboratory of Molecular Carcinogenesis, NIEHS, Res. Tri. Park, NC 27709, Nippon Dental Univ., Tokyo, Japan and Kanagawa Cancer Center, Yokohama, Japan. Carcinogenesis is generally recognized as a multistep process. For a normal cell to evolve into a malignant cell, heritable alterations in multiple genes must be acquired and therefore, neoplastic progression will accelerate if a cell becomes genetically unstable. Although certain tumor cells have increased mutation rates at specific genetic loci, this is not a general observation. In contrast, aneuploidy is a common feature of many cancer cells, and numerical and structural chromosome alterations can contribute to carcinogenesis by both activation of proto-oncogenes and inactivation of tumor suppressor genes. The mitotic apparatus is a target for the carcinogenic activity of several known human carcinogens, such as asbestos and diethylstilbestrol, further supporting a critical role of aneuploidy induction in carcinogenesis. We have proposed that a key step in the neoplastic progression of Syrian hamster embryo cells is the induction of aneuploidy by carcinogens. Since susceptibility of human fibroblast cells in culture to neoplastic transformation by chemical carcinogens is appreciably lower than rodent fibroblasts, it is possible that this differential sensitivity is due to a difference in genetic stability following treatment with chemicals that induce aneuploidy. Therefore, we measured the induction of numerical chromosome changes by four specific aneuploidogens (diethylstilbestrol, 17β-estradiol, colcemid and vincristine) in normal human fibroblasts and Syrian hamster cells. The results indicate that human cells respond to agents which induce aneuploidy to a lesser extent than hamster cells. Although it is clear from cytogenetic studies that tumor cells are aneuploid and karyotypically unstable, it is difficult to quantitate this instability and to determine if it changes during neoplastic progression. One approach to this problem is the introduction of new chromosomes with selectable markers into tumor cells by microcell fusion. These experiments have revealed that transferred chromosomes are more stable in certain tumor cells and this correlates with the general karyotypic stability of the tumor cells. Another determinant of karyotypic instability may be oncogene activation. We have examined a series of Syrian hamster cell lines transfected with the v-Ha-*ras* oncogene and tumor-derived cell lines were markedly altered karyotypically. However, isolated clones expressing the v-Ha-*ras* oncogene prior to injection into animals were not significantly different from control clones. These observations suggest that the observed karyotypic instability is the result of selection *in vivo* for unstable subpopulations rather than a direct result of v-Ha-*ras* oncogene expression.

D 002 GENE AMPLIFICATION IN CANCER/MULTIDRUG RESISTANCE, I.B. Roninson, M. Fukumoto, J.E. Chin, K. Choi, C.-j. Chen, B. Morse, K.E. Noonan, D.H. Shevrin* and R.D. Estensen+, Departments of Genetics and *Medicine, University of Illinois at Chicago, Chicago, IL, 60612 and +Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN 55455-0315.

Gene amplification is a common mechanism for oncogene activation in human cancer. In several types of cancer, amplification of specific oncogenes occurs consistently and appears to correlate with the stage of tumor progression. To study the frequency of gene amplification in cancer, we have developed a modified in-gel DNA renaturation procedure, which detects human DNA sequences amplified as little as 7- to 8-fold. This technique was used to test for the presence of amplified DNA in cell lines or biopsy samples derived from tumors that are not usually associated with oncogene amplification. Gene amplification was detected in two out of 16 cell lines and in two out of 10 surgical specimens of ovarian carcinomas. The amplified DNA sequences in all cases were found to include known cellular oncogenes. In one cell line, gene amplification, involving the c-*myc* gene, became detectable only after the passage of tumor cells in nude mice, suggesting an association of c-*myc* amplification with *in vivo* growth. In both cases of gene amplification detected in ovarian carcinoma tissues, the amplified DNA sequences were found to include the Ki-*ras* oncogene. In one of the tumors, Ki-*ras* was shown to be amplified both in the primary tumor and in different metastatic nodules. No mutations at codons 12 or 61 of Ki-*ras* were detected in these tumors.

Resistance of tumor cells to many lipophilic drugs used in cancer chemotherapy (multidrug resistance; MDR) results from increased expression of the *mdr*1 gene. This gene codes for P-glycoprotein, which appears to function as an ATP-dependent efflux pump, responsible for decreased drug accumulation in MDR cells. Increased expression of *mdr*1 mRNA in some but not all cases is associated with gene amplification. *mdr*1 gene amplification is sometimes accompanied by formation of large circular episomes and specific DNA rearrangements. Although the same *mdr*1 gene is activated in cells selected with different lipophilic drugs, some MDR cell lines are preferentially resistant to their selective agent. In a colchicine-selected MDR cell line, preferential resistance to colchicine results from point mutations, leading to a single amino acid substitution in the *mdr*1 gene. Another human gene, *mdr*2, is homologous to *mdr*1 and linked to it within 330 kb of DNA. Both *mdr* genes are amplified in some MDR cell lines, but only *mdr*1 expression is consistently associated with MDR. Expression of *mdr*1 and *mdr*2 mRNA was analyzed by enzymatic amplification of cDNA sequences. In all tested normal tissues, *mdr*1 was expressed at a higher level than *mdr*2, but in some tumor-derived cell lines only the *mdr*2 gene was expressed. The function of the *mdr*2 gene remains to be determined.

D 003 HST1 ONCOGENE ENCODING AN FGF-RELATED GROWTH FACTOR: ITS PRODUCT, EXPRESSION, CLUSTERING AND COAMPLIFICATION WITH INT2, Teruhiko Yoshida, Kiyoshi Miyagawa, Hiromi Sakamoto, Osamu Katoh, Takashi Sugimura and Masaaki Terada, Genetics Division, National Cancer Center Research Institute, Tsukiji 5-chome, Chuo-ku, Tokyo 104, Japan. HST1, or HSTF1 by human gene nomenclature, was originally identified in our laboratory as a transforming gene in DNA of human stomach cancer. Subsequently, however, other groups also found HST1 in colon cancers, hepatomas and a melanoma. Nucleotide sequence of open reading frame of KS oncogene cDNA identified in a Kaposi's sarcoma was the same as that of HST1. We cloned actively transforming HST1 genomic fragments from a normal individual and also from NIH3T3 cells themselves. Sequence analysis of the cDNA and genomic fragment of HST1 led us to conclude that normal HST1 protein transforms NIH3T3 cells when its expression is deregulated. The amino acid sequence of the HST1 protein has a signal peptide at the N-terminus and is 40-50% homologous to basic and acidic FGFs and to the Int-2 protein. The purified HST1 protein synthesized and processed in silkworm cells was a potent heparin-binding growth factor and promoted the growth of a variety of cells including human vascular endothelial cells and induced anchorage-independent growth of NRK cells. HST1 is rarely expressed in adult cells but is expressed in mid-stage embryos of mice and in some germ cell tumors. Hst-1 is transcribed in mouse teratocarcinoma F9 cells and down regulated upon differentiation into parietal endoderm-like cells; in contrast, Int-2 is expressed only in differentiated F9 cells. These two homologous oncogenes are located close each other within the distance of 40-50 kb on the band q13 of chromosome 11 and coamplified in a variety of cancer cells, most notably in more than 40% of esophageal cancers.

Genomic Stability-II

D 004 MOLECULAR MECHANISMS INVOLVED IN HUMAN B AND T CELL MALIGNANCIES, Carlo M. Croce, M.D., Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, 3420 North Broad Street, Philadelphia, PA 19140. Many human cancers shown nonrandom cytogenetic abnormalities. In particular, chromosome translocations are consistently associated with a variety of hematopoietic malignancies. I will discuss our current understanding of these translocations -- the chromosomes and loci involved in specific translocations, the mechanisms by which the translocations arise, the role of proto-oncogenes activation--as they relate to human malignancies. I will focus on the relatively well-characterized translocations associated with B and T cell lymphomas and leukemias.

D 005 CARCINOGEN-INDUCED HOMOLOGOUS RECOMBINATION BETWEEN DUPLICATED GENES STABLY INTEGRATED WITHIN THE GENOME OF MAMMALIAN CELLS, INCLUDING NORMALLY REPAIRING AND REPAIR-DEFICIENT HUMAN CELLS, J. Justin McCormick, Nitai P. Bhattacharyya, Tohru Tsujimura, Yenyun Wang, and Veronica M. Maher, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824-1316. We have been studying carcinogen-induced homologous recombination in a *tk*$^-$ mouse L cell line, 333M, which contains a single integrated plasmid with duplicated copies of the Herpes *tk* gene, each containing an 8bp *Xho*I site inserted in a different place, and with the *neo* gene located in the intervening sequence. Only by undergoing a productive recombinational event between the two non-functional *tk* genes can a functional gene product be made and the recombinant be selected with HAT medium. With this system, we have determined that the frequency of recombination induced by a series of agents that form multi-ringed DNA adducts, i.e., 1-nitrosopyrene, N-AcO-AAF, 4-NQO, and the 7,8-diol-9,10-epoxide of BP, is linearly related to the number of DNA adducts formed, but some agents are more potent recombinagens than others. We have also shown that UV and X-irradiation, MNNG, and mitomycin C can induce such recombination. The majority of the recombinational events are consistent with non-reciprocal transfer of genetic material, i.e., gene conversion. To examine the mechanisms involved and the role of DNA repair in this process, we have constructed two systems for studying such recombination in several infinite lifespan human cell lines which have normal rates of O^6-alkylguanine DNA repair and nucleotide excision repair or are deficient in one or both processes. One assay system is based on the plasmid carrying duplicated copies of the *Htk* gene described above. The second employs a related plasmid carrying duplicated copies of the gene coding for hygromycin resistance. Using these latter two systems, we have found that nucleotide excision repair-proficient cells have a lower frequency of recombination induced by UV and multi-ringed chemical carcinogens than repair-deficient cells, indicating that repair processes act to prevent recombination. Studies on the effects of O^6-alkylguanine-DNA-alkyltransferase on the frequency of homologous recombination induced by N-methyl-N'-nitro-N-nitrosoguanidine are in progress. (Supported by Grant CA21253 from the National Cancer Institute and by Contract 87-2 from the Health Effects Institute.)

D 006 MAPPING GENES THAT CAUSE DISEASE, Ray White, Howard Hughes Medical Institute and Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, Ut 84132

A map of DNA markers for the human genome, with markers spaced at close intervals on each chromosome, is nearly complete. A strong rationale for the effort to develop such a map has been the promise of locating, by linkage analysis, genes harboring mutations that cause inherited disorders but whose biochemical consequences are not yet understood. As the map becomes more dense and coverage of individual chromosomes becomes more extensive, the pace of such localizations is increasing rapidly; the past two years have seen chromosomal assignments for a number of genes responsible for human diseases. Given sufficient family resources, it is often possible to develop additional markers in the region identified by the initial linkage analysis, to further narrow the target to about 1-2 centimorgans. This genetic distance on average defines a region of only 1-2 million base pairs, a physical distance likely to contain only 3-6 genes that might be expressed in a specific tissue and thus represent candidate loci for the source of the disorder; such transcript regions can be defined and examined in detail. We are applying this strategy in the search for the gene on chromosome 5q that harbors a defect responsible for familial adenomatous polyposis (FAP) and for the locus on chromosome 17 associated with type 1 neurofibromatosis (NF1). The path to isolation of a gene from the 1-2 cM region may include establishing hybrid cell lines that contain smaller and smaller pieces of the human chromosome in question for creation of cosmid libraries, and mapping overlapping cosmids to find sequences that are expressed in certain tissue (such as colon epithelium for FAP and neural crest for NF1). Our strategy for isolation of each of these two genes is similiar but slightly different, because in the case of NF1 we have available to us hybrid cell lines derived from two patients who each carry a balanced translocation in the appropriate region of chromosome 17 and may thus identify the NF1 gene at the breakpoint. Identification and molecular characterization of these and other defective genes will clarify biochemical processes; the FAP gene is of particular interest in that it may shed light on the mechanisms leading to carcinoma of the colon.

DNA Damage, DNA Repair and Mutagenesis

D 007 GENETIC AND BIOCHEMICAL DISSECTION OF MAMMALIAN EXCISION REPAIR,
Jan H.J. Hoeijmakers*, Marcel van Duin*, Geert Weeda**, Christine Troelstra*, Andries Westerveld*, Alex J. van der Eb**, and Dirk Bootsma*, *Department of Cell Biology and Genetics, Erasmus University, Rotterdam and **Dept. of Tumor Virology, University of Leiden, The Netherlands.

DNA repair processes help prevent DNA damage from interfering with essential DNA functions and from inducing mutations, which cause cell death, or contribute to malignancy. One of the major repair systems is the excision repair pathway, operating at lesions (such as UV-photoproducts and bulky adducts), which cause a strong distortion of the normal DNA structure. Inborn defects in this process underly the human syndrome xeroderma pigmentosum (XP), as the consequence of which patients display UV-hypersensitivity and predisposition to skin cancer. Genetic studies of XP-fibroblasts and CHO excision mutants suggest that perhaps 17 or more genes are implicated in nucleotide excision. Genomic DNA transfection to CHO-mutants was used to isolate three correcting human repair genes ERCC-1, -3 and -6.

ERCC-1 was characterized extensively, shown to have aminoacid sequence homology to the yeast excision repair protein RAD10 and parts of the E.coli uvrA and C repair proteins. The ERCC-1 gene overlaps with another gene with unknown function, and is most liklely not mutated in XP.

ERCC-3 specifies a protein of 782 aminoacids, containing putative nuclear location signal, DNA and nucleotide binding domains and acidic stretches, frequently observed in chromatin or histon binding proteins. The gene is strongly conserved, however, a yeast homologue is not (yet) discovered.

ERCC-6 was recently isolated from a secondary UV-resistant transformant of CHO mutant UV61 (generously provided by Dr. L. Thompson, Livermore). This gene has an estimated size of at least 100 kb. Its characterization is in progress.

D 008 GENETIC POLYMORPHISMS IN HUMANS AND THEIR RELATION TO CARCINOGEN-DNA ADDUCT FORMATION, Fred F. Kadlubar, Natl. Center for Toxicol. Res., Jefferson, AR 72079

Epidemiological studies have shown wide variations in human cancer incidences that may arise in part from genetic differences in individual susceptibility to chemical carcinogens. Recent studies with aromatic amines, which are known occupational and environmental carcinogens, have indicated that metabolic differences in humans may serve as critical determinants in the formation of carcinogen-DNA adducts in tissues such as liver, lung, colon, and urinary bladder. Two enzyme systems, that have now been sufficiently characterized to allow for correlations between metabolic phenotype and carcinogen-DNA adduct levels in humans, are the cytochromes P-450 and the acetyltransferases. We have shown that human cytochrome $P\text{-}450_{PA}$, which varies at least 40-fold among individuals, is primarily responsible for aromatic amine N-oxidation; while acetyltransferases, which catalyze the binding of N-hydroxy arylamine metabolites to DNA, have been correlated with differential susceptibility to colo-rectal and bladder cancers. These findings are now being corroborated by measuring both enzyme activities and carcinogen-DNA adduct levels in human tissues.

D 009 GENETIC ANALYSIS OF MAMMALIAN DNA REPAIR, Larry H. Thompson, Christine A. Weber, and Nigel J. Jones, Biomedical Sciences Division, Lawrence Livermore National Laboratory, P.O. Box 5507, Livermore, CA 94550, U.S.A.

We are analyzing human genes that control DNA repair and recombination by complementing rodent mutant lines with either human chromosomes in hybrid cells or with human DNA by transfection. So far our studies point to a high degree of evolutionary conservation of repair gene function between hamster and human. In rodents, eight complementation groups for the nucleotide excision repair pathway are identified: six in CHO cells, a seventh in V79 hamster, and an eighth in mouse lymphoma cells (1). The first five of the complementing repair genes were mapped to human chromosomes 2, 13, 16, and 19 (2).

The human gene *ERCC2* (Excision Repair Cross Complementing for mutants in group 2), was cloned from the cosmid library of a secondary transformant. *ERCC2* restored a normal phenotype in CHO UV5 cells in terms of survival, mutation, and repair incision (3). Restriction fragments of the gene were used to obtain a partially functional cDNA clone from the pcD2 human expression library of H. Okayama. Restriction site mapping and DNA sequencing shows that the gene is ~19 kb and encodes a protein of 760 a.a. This protein has a remarkable 52% identity with the yeast Rad3 protein, which is known to be essential for cellular viability. Analysis of mutations induced in the hamster *ERCC2* locus in CHO cells by various agents suggests that the mammalian gene may also be essential. The properties of one cosmid clone suggest that 5'-flanking sequences may be necessary for stable expression of *ERCC2*.

CHO mutant EM9 has the properties of 2-fold hypersensitivity to ionizing radiation, reduced rate of strand-break repair, 10-fold elevated sister chromatid exchange (SCE), and reduced efficiency of homologous recombination. CldUrd proved to be a highly effective selecting agent in complementation assays. From the cosmid library of an EM9 tertiary transformant, the complementing human gene, *XRCC1* (X-ray Repair Cross Complementing), was obtained. In cosmid-clone transformants of EM9, the *XRCC1* gene efficiently restored resistance to γ-rays, the rate of break rejoining, and a normal level of SCEs. A near full-length, functional cDNA clone of *XRCC1* was obtained, and an open reading frame encoding 633 a.a. was identified by sequencing of cDNA and genomic clones. A restriction site map of an *XRCC1* cosmid shows that the gene is ~33 kb in length. (Work performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under Contract W-7405-ENG-48.)

1. L.H. Thompson et al., **Somat. Cell Mol. Genet.** 14, in press.
2. L.H. Thompson, et al. in Mechanisms and Consequences of DNA Damage Processing, UCLA Symposia on Mol. Biol., New Series, Vol. 83, Eds. E. Friedberg and P. Hanawalt, Liss, New York, in press.
3. C.A. Weber et al. **Mol. Cell. Biol.** 8: 1137-1146, 1988.

Negative Signals or Repression of Cancer (joint)

D 010 MUTATIONS FREQUENTLY FOUND IN PRIMARY HUMAN BREAST TUMORS, Robert Callahan[1], Iqbal Ali[1], Gregory Campbell[2], Craig Cropp[1], Carl Garrett[3], Rosette Lidereau[4], Daniele Liscia[1], Giorgio Merlo[1], [1]Laboratory of Tumor Immunology and Biology, [2]Laboratory of Statistical and Mathematical Methodology, NIH, Bethesda, MD 20892, [3]George Washington University Medical Center, Washington, DC 20037, Centre Rene Hugnenin, St. Cloud, France

We have surveyed a panel of 150 primary human breast tumors and matching lymphocyte DNA preparations and have found that the c myc, c erb B-2, and int-2 genes are amplified in respectively 32%, 10%, and 16% of the tumor DNA's. Amplification of int-2 has a significant association ($P<2x10^{-6}$) with patients which later develop a distant metastasis or local recurrence. The int-2 gene is frequently activated in MMTV induced mouse mammary tumors and is a member of the Fibroblast growth factor (FGF) family of genes. We have examined other markers linked to int-2 on chromosome 11q. In all but one case the BCL-1 locus and hst gene are both coamplified with int-2. Using in situ hybridization on frozen tumor sections and Northern blot analysis, int-2 RNA was detected in tumors in which the gene is amplified. Loss of heterozygosity of specific regions of the human genome has been reported in several types of solid tumors. We have found a loss of heterozygosity of sequences on chromosomes 1, 3p, and 11p in 20%-30% of the tumor DNAs. The most frequently deleted region on chromosome 11p is located between the ß globin and PTH genes. This mutation is associated with histopatholgical grade III tumors ($P<.006$), progesterone receptor negative tumors ($P<.002$) and patients which later develop distant metastasis ($P<.05$). In summary primary human breast tumors contain a heterogeneous array of frequently occuring mutamutations which may contribute to the evolution of the tumor. Some of these mutations may be useful as prognostic indicators in the clinical management of the disease.

D 011 GENES ENCODING SMALL RNAs AND MAMMALIAN CELL GROWTH CONTROL,
Bruce H. Howard, C. Michael Fordis, Kazuichi Sakamoto, Tazuko Howard, Christopher Corsico, and Wolfgang Holter, Laboratory of Molecular Biology, NCI, NIH, Bethesda, MD 20892

Numerous small RNAs are encoded by the mammalian genome. Some of these small RNAs are abundant and have known functions, e.g., the 7SL RNA component of the signal recognition particle, U RNAs involved in mRNA splicing, tRNAs, etc. Additionally there may be many small RNAs of unknown function that occur at low abundance, e.g., the small RNA coded by the first exon of the c-*myc* proto-oncogene. Our interest in the possibility that small RNAs play a role in mammalian growth regulation has led initially to studies on 7SL RNA and the related Alu family of interspersed repetitive sequences. To analyze the consequences of introducing 7SL RNA or Alu gene sequences into HeLa cells, we have used transient transfection assays in which recipient cells are identified by expression of a cotransfected cell surface marker plasmid. Effects on DNA replication in transfected subpopulations are monitored by: i) bromodeoxyuridine or propidium iodide staining followed by fluorescence activated cell sorting (FACS), or ii) ^{3}H-thymidine labeling followed by magnetic affinity cell sorting. In the affinity cell sorting assay both 7SL RNA and Alu sequences exhibit a capacity to suppress ^{3}H-thymidine incorporation; in addition, the FACS assays suggest that, under certain experimental conditions, the 7SL RNA gene may induce aberrant DNA replication. Mutational studies on 7SL RNA suggest that both the signal recognition particle-related function of the intact gene and the Alu moiety within that gene contribute to the inhibitory activity. Mutational studies on several subcloned Alu sequences suggest that the box B component of the polIII promoter and an undecanucleotide sequence homologous to the SV40 origin of DNA replication are important. Possible models to explain these results will be presented and speculations will be offered vis-à-vis their relevance to mammalian cell growth regulation.

Differentiation and Cancer (joint)

D 012 Subtle changes in the expression of the differentiation program (with partial uncoupling) in immortal and tumorigenic human keratinocyte (HaCaT) lines.
Norbert E. Fusenig, Dirk Breitkreutz, Cathy Ryle and Petra Boukamp; Division Differentiation and Carcinogenesis in vitro, German Cancer Research Center Heidelberg, FRG.
Defects in differentiation are commonly associated with epithelial cell transformation, although often analysis of differentiation has been restricted to a few parameters and conventional culture conditions. We observed that many transformed human keratinocyte lines exhibited nearly complete differentiation in organotypical culture and particularly in transplants. However, morphological differentiation and synthesis of respective markers were not always strictly correlated: e.g. the immortalized HaCaT cells readily expressed the epidermal keratin pair K1 and K10 (already in submerged cultures, unlike normal cells) but stratification was gradually lost. In contrast, some malignant HaCaT clones (transfected with Ha-ras-oncogene) had restored stratification in vitro while K1 and K10 was slightly reduced. Thus, full differentiation capacity and responsiveness to environmental signals might be better evaluated in transplants or equivalent culture systems: regular epithelia were formed in transplants of HaCaT and respective tumorigenic cells, while spatial expression of K1 and K10 and its coordination was altered, but more severely in HaCaT clones. In conclusion i) a high degree of differentiation is compatible with immortalization and malignancy, ii) subtle changes are only detectable under "permissive" conditions, iii) differentiation parameters can dissociate and iv) some aspects might even be improved e.g. by ras-transfection-mediated changes. Environmental modulation (at varying degrees) was also observed in other in vitro transformed or human SCC lines stressing the relevance of such "bio-assays".

D 013 ROLE OF TGF-B IN CONTROL OF CELL DIFFERENTIATION, Michael Sporn, David Danielpour, Fabienne Denhez, Pamela Dillard, Andrew Geiser, Adam Glick, Sonia Jakowlew, Seong-Jin Kim, Paturu Kondaiah, Robert Lechleider, Takafumi Noma, Lalage Wakefield, and Anita Roberts, Laboratory of Chemoprevention, National Cancer Institute, Bethesda, Maryland 20892.
At least 5 unique molecular species of TGF-B are now known to exist in mammalian, avian, and amphibian species. Distinct regulatory elements for the genes of each of these TGF-B's are presently being defined. The actions of the various TGF-B's in control of cell differentiation and other critical cellular functions will be discussed, with emphasis on the role of promoter sequences in control of gene expression. The importance of understanding of the physiology of various latent forms of TGF-B will also be considered.

Development and Cancer

D 014 REGULATORY GENES THAT CONTROL DETERMINATION OF VERTEBRATE MYOGENIC CELL LINEAGES, Deborah F. Pinney, Sonia H. Pearson-White, Brian Lathrop, and Charles P. Emerson, Jr., Department of Biology, University of Virginia, Charlottesville, Virginia 22901 The mouse embryonic cell line, C3H10T1/2, is multipotential and can be converted at high frequencies to stable, clonal lineages of myogenic, adipogenic and chondrogenic cells by brief exposure to the DNA hypomethylating agent, 5-azacytidine.[1] These observations suggested that 5-azacytidine activates, by a DNA hypomethylation mechanism, one or a few regulatory genes that control the establishment of these cell lineages. DNA transfection approaches have been used to identify two regulatory genes that can direct the myogenic conversion of 10T1/2 cells. 10T1/2 cells transfected with a cosmid library of human genomic DNA segments yields clonal primary and secondary myogenic transfectants.[2] Experiments are in progress to characterize the structure and function of this transfected myogenic gene segment, myd. A second myogenic gene, MyoD1,[3] is activated in 5-azacytidine-converted and myd-transfected myogenic cell lines. MyoD1 also can induce clonal myogenic conversion and promote the differentiation of 10T1/2 cells when transfected as a cDNA transcribed by a heterologous β-actin or a SV-40 promotor. MyoD1 is a nuclear-localized protein,[4] and bacterially-expressed MyoD1 protein has sequence-specific DNA binding activity, suggesting MyoD1 regulates muscle-specific genes. The roles of myd and MyoD1 in myogenic lineage determination and differentiation will be discussed.

[1]Konieczny and Emerson (1984). Cell 38, 971.
[2]Pinney. et al (1988). Cell 53, 781.
[3]Davis, et al (1987). Cell 51, 987.
[4]Tapscott, et al (1988). Science 242, 405.

Genetic Mechanisms in Carcinogenesis and Tumor Progression

Tumor Promotion and Progression

D 015 RESPONSE MODIFICATION IN OXIDANT TUMOR PROMOTION OF MOUSE EPIDERMAL CELLS JB6. P. Cerutti, G. Krupitza, R. Larsson and P. Amstad, Department of Carcinogenesis, Swiss Institute for Experimental Cancer Research-1066 Epalinges/Lausanne, Switzerland.

An early step in carcinogenesis involves hereditary changes in the DNA sequence. Typically, these alterations result in a "response modification" of the affected cell to extracellular signals and cytotoxic agents which allows its selection and clonal expansion by a tumor promoter to an usually benign tumor. For malignancy a potentially malignant chromosomal aberration may be required as an additional rare event (1).

This concept is illustrated for the case of the oxidant tumor promotion of mouse epidermal cells JB6. Because of their ubiquitous occurrence oxidants may represent "natural" promoters. While being toxic they also trigger pathophysiological reactions which resemble those induced by growth- and differentiation factors. For example, active oxygen (AO) causes S6-phosphorylation, the activation and translocation of protein kinase C and the transcriptional activation of growth competence genes in JB6 cells. For the induction of the immediate early gene c-fos the post-translational poly ADP-ribosylation of chromosomal proteins, in particular of fos-protein itself, plays a role. Inhibition of ADPR-transferase by benzamide both suppressed the poly ADP-ribosylation of fos-protein and prevented the transcriptional induction of c-fos by AO. We propose that c-fos is induced by a de-repression mechanism. In JB6 (clone 41) cells growth promotion by AO appears to be due to a "response modification" involving an increase in resistance to AO-cytotoxicity and changes in the pathophysiological reactions which are induced by AO.

(1) P. Cerutti, Carcinogenesis 9, 519-526 (1988).
(2) P. Cerutti, R. Larsson, G. Krupitza, D. Muehlematter, D. Crawford and P. Amstad in: "Oxy-Radicals in Molecular Biology and Pathology" (eds. P. Cerutti, I. Fridovich and J. McCord) pp. 493-507, Alan R. Liss, Inc. (1988).

D 016 **CLONAL DOMINANCE OF PRIMARY TUMORS BY METASTATIC SUBPOPULATIONS: GENETIC ANALYSIS AND BIOLOGICAL IMPLICATIONS** Carol Waghorne, B. Korczak, Martin Breitman and R.S. Kerbel, Mount Sinai Hospital Research Institute, Toronto, Ontario, Canada M5G 1X5

Large numbers of independent clonotypic markers were introduced into a mouse mammary adenocarcinoma (called SP1) by transfection of the cells with pSV_2neo or infection with the retrovirus vector, ΔpΔeMoTN. Each transfectant (or infectant) thus bears a unique genetic marker because of the random integration of the integrated plasmid or proviral DNA molecules. Southern analysis can therefore be used as a "lineage tracer" to track the fate of individual clones in vivo after injection of potentially complex mixtures of cells. One of the most striking findings using this experimental approach was evidence for rapid and extensive clonal selection: thus even when as many as 10^4 or 10^5 uniquely tagged SP1 clones were pooled and the cells injected subcutaneously into syngeneic CBA/J mice, the resultant primary tumors analyzed six weeks later were found to be effectively dominated by the progeny of less than 5 to 10 clones. The identity of these "dominant" clones was affected by the initial ratio of metastatic to non-metastatic cells. For example, if the ratio was in the range of 1:50, the minor metastatic subpopulation always came to dominate the primary tumor mass at late stages of tumor growth. This occurred despite any evidence to indicate the metastatic cells had a shorter population doubling time compared to their non-metastatic counterparts. These results suggest that clonal (cell-cell) interactions or local growth factor conditions in the primary tumor can impart a selective growth advantage to cryptic metastatic cells. As a result, late stage, advanced primary tumors may evolve to become genotypically and phenotypically similar to their distant metastases. The implications of this finding, and possible mechanisms will be discussed.

D 017 GENES AND MECHANISMS INVOLVED IN MALIGNANT CONVERSION. Stuart H. Yuspa, Henry Hennings, Dennis Roop, James Strickland, David A. Greenhalgh, LCCTP, NCI, NIH, Bethesda, MD 20892. The elucidation of the cellular and molecular events involved in progressive stages of malignant transformation has been enhanced by the development of new in vivo and in vitro model systems. In the model of chemically-induced mouse skin tumors, multiple benign squamous papillomas precede the development of an occasional squamous cell carcinoma. The incidence of carcinomas can be substantially enhanced by treating papilloma-bearing mice with mutagens such as urethane, nitroquinoline-N-oxide or cisplatinum suggesting that a distinct genetic event catalyzes malignant conversion. The malignant phenotype is characterized by a marked reduction in the transcription of specific markers of epidermal differentiation, a pattern which is useful for the early diagnosis of malignant conversion. In epidermal cell cultures, the benign phenotype can be obtained by introducing the v-ras oncogene into primary cells by a defective retrovirus. Subsequent treatment of recipient cells with chemical mutagens produces foci with a malignant phenotype in vitro. Benign epidermal tumor cells in culture are good recipients for exogenous DNA to detect genes involved in malignant conversion. Such studies reveal that transforming constructs of the fos oncogene induce malignant conversion whereas myc and adenovirus E1A are not converting genes. Malignant tumors induced by fos transfection do not express differentiation-specific epidermal markers and secrete transin, a protease characteristic of malignant skin tumors and a product of a gene which is regulated by fos-AP-1. These studies with fos implicate a limited number of genes in the malignant conversion of squamous tumors.

1. Hennings, H., Shores, R., Wenk, M., Spangler, E. F., Tarone, R., and Yuspa, S. H.: Malignant conversion of mouse skin tumours is increased by tumour initiators and unaffected by tumour promoters. Nature 304: 67-69, 1983.
2. Roop, D. R., Krieg, T. M., Mehrel, T., Cheng, C. K. and Yuspa, S. H.: Transcriptional control of high molecular weight keratin gene expression in multistage mouse skin carcinogenesis. Cancer Res. 48: 3245-3252, 1988.
3. Greenhalgh, D. A. and Yuspa, S. H.: Malignant conversion of murine squamous papilloma cell lines by transfection with the fos oncogene. Molecular Carc., in press.

Angiogenesis and Angiogenic-Like Peptides (joint)

D 018 FIBROBLAST GROWTH FACTOR: BIOLOGICAL ROLE IN EARLY EMBRYONIC DEVELOPMENT AND CELL TRANSFORMATION, D. J. Gospodarowicz, Cancer Research Institute, The University of California Medical Center at San Francisco, CA 94143 Basic fibroblast growth factor (FGF) and acidic FGF are two closely related multifunctional peptides which interact with the same cell surface receptor. They control proliferation, differention, and various other cellular functions in cells derived from the mesoderm and the neuroectoderm. The biological effect of FGF which could contribute to normal development in early embryos and neonates will be reviewed. Also reviewed will be the possible implication of FGF in neoplastic transformation, since uncontrolled expression of the bFGF gene has been shown to result in autonomous cell proliferation and the expression of the transformed phenotype.

D 019 BASIC FIBROBLAST GROWTH FACTOR EXPRESSSION IN TRANSFORMED CELLS, Michael Klagsbrun and Snezna Rogelj, Departments of Surgery and Biological Chemistry, Children's Hospital, Harvard Medical School, 300 Longwood Ave, Boston, MA 02115

Basic fibroblast growth factor (bFGF) is a cell-associated growth factor. The lack of bFGF secretion into conditioned medium is consistent with the apparent lack of a consensus signal peptide in the open reading frame of the bFGF gene. Many normal cells, e.g. endothelial cells, synthesize bFGF. However, it is possible to convert bFGF into a transforming protein by transfecting cells with bFGF cDNA to which a signal peptide sequence is fused to the amino-terminus of the coding region. NIH 3T3 cells expressing signal peptide-bFGF (sig-bFGF) display a highly transformed phenotype in vitro. They are highly tumorigenic in vivo and produce tumors in syngeneic animals at a rate and size comparable to tumors produced by ras-transfected cells. In contrast, NIH 3T3 cells transfected with native bFGF cDNA grow as a monolayer and do not produce tumors in syngeneic mice even if they express 10 times as much bFGF as do the sig-bFGF expressing cells. Despite addition of a signal peptide, there is no evidence that the bFGF is being secreted into the medium of sig-bFGF expressing cells. Antibodies that neutralize bFGF have no effect on the transformed phenotype of cells expressing sig-bFGF. Cross-linking studies with ^{125}I-bFGF show that there is a lack of bFGF binding sites on the surface of sig-bFGF expressing cells. This is in contrast to parental NIH 3T3 cells and NIH 3T3 cells expressing native bFGF which do have cell surface bFGF binding sites. From these results we postulate that for bFGF to be transforming it must not only be expressed but also translocated to a site where it can interact with its receptor and that transformation of the cells by bFGF occurs via an internal autocrine loop. In other studies we find that oncogenic transformation of cells appears to be correlated with increased levels of bFGF synthesis. 3T3 cells and RAT-1 cells transformed with the oncogenes ras, neu and sis synthesize 5-10 times as much cell-associated bFGF as do the parental cell lines. Some of the bFGF produced by these cells is a higher molecular weight form of about 23,000 Daltons compared to the usual bFGF molecular weight of 18,000 Daltons. The effect of increased bFGF levels and higher molecular weight forms on the transformed phenotype and tumorigenic potential of oncogene-transformed cells is being presently analyzed.

Tumor Invasion and Metastases

D 020 ADHESION AND INVASION IN LYMPHOMA METASTASIS, Folkert F. Roossien, Geertje La Rivière, John Collard and Ed Roos, Division of Cell Biology, The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX Amsterdam, The Netherlands.

Certain lymphoma cells metastasize widely, in particular to the liver, which is massively and diffusely invaded. Since activated T-lymphocytes invade hepatocyte cultures, we hypothesized that lymphoma invasiveness is due to the inherent invasive potential of lymphocytes. Indeed, hybrids generated by fusion of non-invasive, non-metastatic lymphoma cells with activated T-lymphocytes, are highly invasive and metastasize widely. Upon segregation of relevant chromosomes, invasiveness is partly or completely lost. Using (human x mouse) hybrids we have shown that expression of a gene located on human chromosome 7 is required. Thus, a highly malignant phenotype can be obtained by inappropriate expression of normal properties. In contrast to other types of (normal x tumor) hybrids, tumorigenicity is not suppressed in T-cell hybrids, indicating that "anti-oncogenes" are not expressed in activated T-cells. The activated ras oncogene also induces lymphoma-specific metastatic behaviour in lymphoma cells and thus apparently mimics the activity of the normal gene(s) involved.

To invade tissues, lymphoma cells have to adhere to cells and subsequently invade between cells, e.g. hepatocytes in the liver. Adhesion is mediated by multiple surface molecules. This includes LFA-1, since invasion into hepatocyte cultures is inhibited to 30% of controls by anti-LFA-1 antibody. Invasion into fibroblast monolayers is reduced even more. We have recently generated three distinct LFA-1-deficient mutant T-cell hybrids, the invasiveness of which in hepatocyte and fibroblast cultures is comparable to parental cells in the presence of anti-LFA-1 antibody. The mutants have normal growth rates and are tumorigenic but their metastatic potential is greatly attenuated. This indicates that sufficiently strong adhesion to cells in tissues is required for efficient metastasis formation.

Invasion into hepatocyte and fibroblast cultures as well as metastasis in vivo is inhibited by pretreatment of lymphoma cells with pertussis toxin. This implies that an extracellular ligand generates a signal inducing invasion. Evidence will be presented that this ligand is an autocrine motility factor. In addition, exogeneous cytokines were found to influence invasiveness. The invasive machinery induced in activated T-lymphocytes apparently includes a set of receptors and cytokines involved in motility. The effect of pertussis toxin, the activated ras oncogene and the involvement of autocrine motility factors have also been documented for other tumors indicating that lymphomas are a good model for invasion and metastasis mechanisms in general.

Late Addition

D 021 THE GENETIC CONTROL OF RECOMBINATION BETWEEN REPEATED SEQUENCES IN YEAST. Rodney J. Rothstein, John W. Wallis, Jr.* and W. Lane Arthur , Department of Genetics and Development, Columbia University College of Physicians and Surgeons, New York, NY 10032.

We have been studying the mechanism of recombination between repeated sequences in the yeast, *Saccharomyces cerevisiae*. There are approximately 35 copies of *Ty*, a retrotransposon that is flanked by LTR repeats called δ sequences. Approximately 200 solo δ sequences are scattered throughout the haploid yeast genome and are thought to result from δ–δ recombination events that delete a *Ty* element. Specifically, we investigated recombination between 5 δ sequences that surround *SUP4*, a tyrosine tRNA locus in yeast, which results in deletions and inversions at a frequency of 10^{-7} and are dependent on the pleiotropic recombination and repair gene, *RAD52*. To investigate other genes that may control the frequency of rearrangements at *SUP4*, mutations were sought that altered the deletion frequency. One EMS-induced mutation was isolated that increased rearrangements almost 10-fold. It was named *edr1* for enhanced *d*elta *r*ecombination. A wild-type clone that complements the defect was isolated and a null mutation was created by gene replacement. Deletions at *SUP4* increased 40- to 100-fold compared to wild-type cells. In addition, several pleiotropic phenotypes were noted. Cells grew 2 times more slowly than wild-type and exhibited an altered mitotic index in log phase--between 60-70% of the cells were in the S-G_2 phase of the cell cycle as opposed to 35% in wild-type. Diploid formation was reduced and the few diploids that did form failed to sporulate.

The cloned DNA was sequenced and computer analysis revealed that the *EDR1* gene encodes an approximately 500 amino acid long open reading frame that is strikingly similar to the *TopA* gene of *E.coli*, which encodes the ω protein. This putative protein is not homologous to the two known yeast topoisomerase genes, *TOP1* and *TOP2*, or to any other known eukaryotic topoisomerase. Double mutants of *edr1* with either *top1* or *top2* result in a synergistic growth defect suggesting that these genes encode functions that can compensate for one another. Studies are in progress to show that *EDR1* indeed encodes a functionally similar protein.

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Genetic Mechanisms in Carcinogenesis and Tumor Progression

Genomic Stability and Damage

D 100 COMPLEMENTATION OF DNA REPAIR DEFECT IN XERODERMA PIGMENTOSUM CELLS: CHROMOSOMAL ASSIGNMENT OF THE REPAIR GENE, Raghbir S. Athwal and G. Pal Kaur, Department of Microbiology and Molecular Genetics, New Jersey Medical School, Newark, NJ 07103.

Complementation of the repair defect in xeroderma pigmentosum cells of complementation group A (XP-A) was achieved by the transfer of intact human chromosome 9. For this purpose a set of mouse/human (M/H) hybrid cell lines each containing a single different human chromosome, marked with Ecogpt, were used as a source of donor chromosome. Chromosome transfer to XPTG-1, an hypoxanthine-guanine-phosphoriboysltransferase (hgprt) deficient mutant of SV40 transformed XP-A cells, was achieved by microcell fusion and selection for Ecogpt. Chromosome transfer clones of XPTG-1 cells each containing a different donor human chromosome were analysed for complementation of sensitivity to UV irradiation. Of all recovered clones, each for the transfer of a different human chromosome, only XPM24-1 and XPM24-2, containing chromosome 9, exhibited increased levels of resistance to UV irradiation. Since our recipient cell line XPTG-1 is hgprt deficient, cultivation of XPM24 clones in medium containing 6-thioguanine (6TG) permits selection of cells for loss of transferred chromosome 9. Analysis of XPMTG clones, isolated for growth in 6-TG, showed correlated loss of UV resistance, with loss of the marked chromosome, confirming the presence of a repair gene for XP-A on chromosome 9. Availability of M/H monochromosomal hybrids containing dominantly marked chromosomes and hgprt$^-$ repair deficient XP-A cell lines made it possible to identify a complementing chromosome. The data on the regional assignment of the repair gene and significance of partial complementation in relation to gene dosage will be discussed.

D 101 CARCINOGEN-INDUCED GENE REARRANGEMENT - ANALYSIS OF GENE STRUCTURE, CHROMATIN CONFORMATION, AND DNA METHYLATION, Frederic G. Barr[+*], Sridharan Rajagopalan[+], and Michael W. Lieberman[+], [+]Department of Pathology, Fox Chase Cancer Center, Philadelphia, PA 19111 and [*]Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA 19104.

We have previously demonstrated that the hamster thymidine kinase (TK) gene can be activated by the chemical carcinogen N-methyl-N'-nitro-N-nitrosoguanidine and that the region far-5' to the TK gene is rearranged in 20% of the TK-expressing variants (Barr et al., Mol. Cell. Biol. 6:3023-3033, 1986). The breakpoint of the rearrangement is localized by Southern blot analysis in one cell line to a 750 bp fragment 6.7 kb 5' to the origin of transcription and in three cell lines to a 400 bp fragment 5.8 kb 5' to the origin. Under stringent hybridization conditions, the latter 400 bp fragment corresponds to a single copy sequence. These rearrangements consist at least partly of a deletion of wild type sequences 5' to these breakpoints. Southern blot analysis of the region near the origin of transcription in the rearranged TK genes demonstrates demethylation of deoxycytidine residues, a DNase I-sensitive chromatin conformation, and a DNase I hypersensitive site. Although the domain of demethylation extends into the vicinity of the rearrangement breakpoint region, the rearranged region does not have a DNase I-sensitive chromatin conformation nor DNase I hypersensitive sites.

D 102 DIFFERENCES IN ACTIVATION OF PROTOONCOGENES IN SPONTANEOUS AND CHEMICALLY INDUCED LIVER TUMORS FROM A/J AND C3H MICE, S.A. Belinsky,T.R. Devereux and M.W. Anderson, NIEHS, RTP, NC 27709

The activation of protooncogenes in either spontaneous or chemically induced liver tumors was investigated using strains of mice which exhibited either a high (C3H) or low (A/J) background incidence for liver neoplasia. Mice were treated for 7 weeks (3 times/wk, i.p.) with either 4-(N-methyl-N-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK, 50 mg/kg), nitrosodimethylamine (NDMA, 3 mg/kg) or vehicle (trioctanoin) and sacrificed over a 10 month period following cessation of carcinogen administration. This resulted in a 18, 23, and 59% incidence of hepatic tumors in A/J mice treated with vehicle, NNK or NDMA, respectively and a 45, 60, and 60% incidence of hepatic tumors in C3H mice treated similarly. Following transfection of high molecular weight DNA onto NIH/3T3 mouse fibroblasts, transforming genes were observed in only 2 of 5 and 0 of 8 of the liver tumors from A/J mice treated with NNK and NDMA, respectively. The amplification of sequences homologous to either K-,H-,N-ras or the raf gene was not observed in Southern blots from A/J liver DNA transfectants. In contrast, transformation of NIH/3T3 fibroblasts was observed in 4 of 6 and 3 of 5 liver tumors from C3H mice treated with vehicle or NNK. Southern blot analysis indicated that the transforming gene present in all of these tumors was the H-ras. The specific mutations in the H-ras genes from both spontaneous and chemically induced tumors will be identified by amplification of the H-ras gene via the polymerase chain reaction followed by direct sequencing. A comparison of mutation profiles in spontaneous and chemically induced liver tumors from C3H mice should aid in determining the role of DNA methylation in the activation of the H-ras gene in this highly sensitive mouse strain.

D 103 HOMOLOGOUS RECOMBINATION BETWEEN DUPLICATED THYMIDINE KINASE (tk) GENES STABLY INTEGRATED WITHIN THE GENOME OF NORMALLY REPAIRING AND REPAIR-DEFICIENT HUMAN CELLS, Nitai P. Bhattaharyya, Veronica M. Maher, and J. Justin McCormick, Carcinogenesis Lab, Michigan State Univ, East Lansing, MI 48824-1316

We have been studying carcinogen-induced homologous recombination in a tk^- mouse L cell line (333M) which contains a single integrated plasmid with duplicated copies of the Herpes tk gene, each containing an 8bp XhoI site inserted in a different place, and with the neo gene located in the intervening sequence. The frequency of recombination induced by agents that form multi-ringed DNA adducts is a function of the number of adducts formed, but some agents are more potent recombinagens than others. We transfected this plasmid (pJS-3) into three tk^- infinite lifespan human cell lines which have normal rates of O^6-alkylguanine DNA repair and nucleotide excision repair or are deficient in one or both processes. Southern blot analysis of DNA from tk^+ stable transfectants indicated the presence of 1-2 intact plasmids integrated into the chromosome. The rate of spontaneous recombination in the various cell lines ranged from 0.15×10^{-6} to 3.5×10^{-6} cells per cell generation. Southern blot analysis of DNA from these tk^+ cells showed that in all cases one of the Htk genes had become wild type (XhoI-resistant). The majority of the spontaneous recombinants retained the Htk gene duplication information. The frequency of homologous recombination induced by UV or by 1-nitrosopyrene was lower in cells which are proficient in nucleotide excision repair than in the repair-deficient cells, indicating that this repair acts to prevent carcinogen-induced recombination. This research was supported in part by Grant CA 21253 from the National Cancer Institute and by Contract 87-2 from the Health Effects Institute (HEI).

D 104 HUMAN RETINOBLASTOMA GENE: LONG-RANGE MAPPING AND ANALYSIS OF A HOMOZYGOUS PARTIAL DELETION MUTANT, Robert Bookstein, Eva Y.-H. P. Lee and Wen-Hwa Lee, *Department of Pathology M-012 and Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093.*

Mutational inactivation of the retinoblastoma (RB) gene is considered a fundamental event in the formation of several types of human cancers. A substantial proportion of RB gene mutations are partial or complete deletions that extend an unknown distance beyond one or both ends of the gene. To provide a framework for measuring the extent of these deletions, we have constructed a long-range restriction map of *Sfi*I sites spanning 850 kb around the RB gene. This map was applied in a molecular analysis of RB gene deletion in breast cancer cell line MDA-MB468. A previous study of this cell line demonstrated deletion of the entire RB gene except for exons 1 and 2 (E. Y.-H. P. Lee, H. To, J.-Y. Shew, R. Bookstein, P. Scully and W.-H. Lee, Science 241: 218-221, 1988). Genomic clones containing the deletion junction were isolated from a library made from MDA-MB468 DNA. A probe obtained from the far side of the the deletion junction was used to localize and clone the unknown 3' endpoint, demonstrating that the chromosomal mutation in this case was a simple deletion spanning 200 kb. Sequence analysis of the deletion junction indicated a conservative deletion with no loss or gain of nucleotides. The deletion endpoints had no sequence homology to each other or to any repetitive sequence family such as *Alu*, so the recombination event was illegitimate. Structural analysis of this and other RB gene deletions is important for understanding molecular mechanisms of recessive oncogenesis.

D 105 ESTIMATION OF THE MUTAGENICITY OF METAL CARCINOGENS BY A NEW ASSAY USING CELLS INFECTED WITH A CONDITIONALLY DEFECTIVE RETROVIRUS, Susanna M. Chiocca, Neal W. Biggart, and Edwin C. Murphy, Jr., Department of Tumor Biology, Section of Molecular Virology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030. We have developed a new assay for DNA damage by potential mutagens and applied it to the study of metal carcinogens. The assay depends on the mutagen-induced reversal of a conditional defect in the expression of the v-mos protein in cells infected with the MuSVts110 retrovirus. The defect is viral and affects the growth temperature at which the splicing of the viral RNA to form the mos product mRNA can occur. Reversal of the defect allows "revertants" to grow as transformed foci at growth temperatures formerly nonpermissive for cell transformation. Two classes of metal-induced revertants have been observed: Class I revertants were induced by nickel salts and a control mutagen, NMU. In these cells, splicing of MuSVts110 RNA was no longer growth temperature regulated. The alteration in splicing efficiency could be transferred to NIH3T3 cells with the viral DNA, and splicing of RNA from a "tagged" and therefore distinguishable MuSVts110 DNA transfected into nickel revertant cells retained its original growth temperature sensitivity, arguing that the nickel-induced mutation was cis with respect to the viral DNA. Class II revertants were induced by chromium and cadmium salts. In these cells, frameshift mutations aligning the MuSVts110 gag and mos genes into a single open reading frame appeared to have occurred, allowing the synthesis of a large gag gene-mos gene fusion polypeptide from the unspliced viral transcript. The precise nature of the damage done to the MuSVts110 sequence in each of the revertants is being explored by RNAse A mapping and PCR.

D 106 MUTAGENESIS BY NICKEL COMPOUNDS, Nelwyn T. Christie and Donna M. Tummolo, New York University Medical Center, Institute of Environmental Medicine, Box 817, Tuxedo, NY 10987. Numerous compounds of Ni(II) are strongly carcinogenic as evidenced by epidemiological studies, animal tumor model systems, and by in vitro cell transformation. Although Ni(II) was reported nonmutagenic in many assays, several laboratories have reported a strong comutagenic activity of this ion in combination with other mutagens. The reason for its weak mutagenic response may be the inability of many assays to detect deletions or rearrangements. We have evaluated the mutagenic response of soluble and relatively insoluble, particulate compounds of Ni(II) in a mammalian mutagenesis assay that is apparently capable of detecting deletions induced by x-rays. The assay system consists of the gpt gene of E. coli stably integrated into the genome of a V79 hamster cell line deficient for the endogenous HGPRT locus, the g12 cell line. In contrast to many other similar systems the spontaneous mutation frequency for this cell is very low, 13 to 25 mutants per 10^6 clonable cells. The low rate of spontaneous mutation of this cell line indicates the absence of a high rate of deletions or rearrangements at the site of integration of the pSV2gpt plasmid and justifies its use to study the induction of deletions or rearrangements by Ni(II). While the mutation rate with soluble $NiSO_4$ is only 2 to 3 times over the background spontaneous rate, the mutation rate with particulate, crystalline NiS (crNiS) ranges from 300 to 1000 mutants per 10^6 clonable cells. The mutagenic response of the g12 cells to crNiS is similar in magnitude to that of the highly mutagenic alkylating agent, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). This study represents the first demonstration that any compound of Ni(II) is capable of producing a strong mutagenic response.

D 107 DELETION OF HETEROCHROMATIN AS A MECHANISM OF NICKEL CARCINOGENESIS, Costa, Max and Kathleen Conway, Institute of Environmental Medicine, New York University Medical Center, 550 First Avenue, New York, NY 10016
Acute treatment of mouse and Chinese hamster cell lines with nickel compounds selectively induces damage in heterochromatic regions of chromosomes. Elevating extracellular magnesium levels inhibits nickel-induced transformation and also inhibits heterochromatic damage to a greater extent than euchromatic damage. A large proportion of the nickel-induced heterochromatic damage is found on the long arm of the X-chromosome in Chinese hamster ovary cells. Male or female Chinese hamster embryo cells were tested for transformation to anchorage-independent growth following exposure to crystalline NiS, $NiCl_2$ or MCA. Eight of 13 male cultures tested grew in agar following nickel exposure while only 2 of 10 female cultures were able to grow in soft agar. Subclones were obtained from the agar colonies and 5 of the male nickel-transformed clones grew, upon reculturing, to high saturation densities and demonstrated a dense, criss-crossed pattern of cell growth. None of the two agar positive female clones grew well in culture. All (4/4) of the MCA-treated cultures tested grew in agar; 2 of these were female. These results suggest that male Chinese hamster cells transform at a considerably higher frequency than female cells following exposure to nickel. When G- and C-banding were performed on the nickel-transformed male lines, 4 of 5 of the cell lines exhibited a deletion of all or part of the heterochromatic long arm of the X-chromosome as the predominant aberration. These data suggest that nickel may, by interacting with heterochromatin in the long arm of the X-chromosome, induce a loss of a gene that suppresses the transformed phenotype. We are currently testing this hypothesis.

D 108 CAFFEINE ACTION ON CELL CYCLE CONTROL AFTER DNA DAMAGE, C.Stephen Downes, Stephen R.R.Musk, James V. Watson* and Robert T. Johnson, Department of Zoology, Cambridge University, Cambridge CB2 3EJ, UK; and *Department of Clinical Oncology and Radiotherapeutics, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK.
In mammalian cells where DNA replication is delayed by polymerase inhibitors or by DNA damage, caffeine allows condensation of chromatin to occur even though replication is not complete[1,2]. The nuclear structures so formed are analogous to the prematurely condensed S-phase chromosomes that can be induced by fusing replicating cells with mitotic cells[3]. It has been reported[1,4] that caffeine-induced S-phase condensation is likewise premature; in BHK hamster cells supposedly arrested at the start of S-phase and given caffeine, condensed chromosomes appear well before the normal time of mitosis. Caffeine is therefore supposed to act by accelerating the mitotic condensation cycle, as well as uncoupling it from replication. We find, however, that BHK cells are not well synchronised by the procedure previously used. With better synchronisation, or other cells, caffeine in replication-arrested cells restores the normal timing of mitotic condensation, and uncouples it from replication, but does not accelerate condensation.
1. Schlegel, R., and Pardee, A.B. (1986) Science 232, 1264-1266.
2. Musk, S.R.R., Downes, C.S., and Johnson, R.T. (1988) J. Cell Sci. 90, 591-599.
3. Johnson, R.T., and Rao, P.N. (1970) Nature 226, 717-722.
4. Schlegel, R., and Pardee, A.B. (1987) Proc. natn. Acad. Sci. U.S.A. 84, 9025-9029.

D 109 CHARACTERIZATION AND LONG RANGE MAPPING OF AMPLIFIED DNA FROM DOUBLE MINUTES IN TUMORIGENIC MOUSE 3T3 CELLS

Steven S. Fakharzadeh, Eric K. Hoffman, Maureen E. Murphy, and Donna L. George, Department of Human Genetics and Howard Hughes Medical Institute, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

We are studying a tumorigenic derivative of mouse 3T3 cells that stably maintains double minutes (DMs), small acentromeric chromatin particles that result from gene amplification. The persistence of DMs in this 3T3-DM cell line is consistent with the hypothesis that a gene whose overexpression provides these cells with some growth advantage is amplified and overexpressed. However, no known oncogene is amplified in these cells.

Coincident with functional studies designed to identify a potentially new oncogene, we are exploring the structure and organization of the amplified domain in the 3T3-DM cell line. CpG-rich islands of DNA tend not to be randomly distributed in the genome but generally cluster at the 5' ends of genes, including both upstream and exon-encoding regions. Based on this concept, we describe a multi-faceted approach designed to generate probes for (1) constructing a long range map of the amplified domain and (2) identifying transcription units within it that will serve as substrate for functional studies.

D 110 BASE-SEQUENCE ANALYSIS OF AMES/*SALMONELLA* MUTATIONS: A LOOK AT THE SPECTRUM OF FRAMESHIFT CHANGES GENERATED BY B[a]P AND AROMATIC-AMINE CARCINOGENS, James S. Felton, James C. Fuscoe*, and Rebekah Wu, Biomed. Sci. Div., Lawrence Livermore National Laboratory, Livermore, CA 94550 and *Center for Environ. Health, Univ of Conn., Storrs, CT, 06268.

This report is an investigation of the specific sequence changes in the DNA of *Salmonella hisD3052* revertants induced by a set of frameshift specific mutagens. They include B[a]P, Aflatoxin B_1, and the cooked-food mutagens, IQ, MeIQ, and PhIP. Sequencing was accomplished by cleaving the *Salmonella* DNA with restriction enzymes Sau3A, Eco R1, and AluI to give a 650 bp fragment. The fragments, once size fractionated, were ligated to the bacteriophage vector M13mp8. After transformation into *E. Coli*, the recombinants were screened with a nick-translated *hisD* gene probe and the isolated single standed DNA sequenced using the Sanger/dideoxynucleotide chain termination method. In the strain TA1538, all IQ (13), MeIQ (3), PhIP (5), and Aflatoxin B_1 (3) induced revertants isolated had a 2 base (CG dinucleotide) deletion situated 10 bases upstream from the original *hisD3052* mutation (C deletion). In contrast, 9 of 24 revertants induced by B[a]P had extensive deletions varying from 8 to 26 nucleotides. The other 15 revertants had the same CG deletion at the same location as described for the other mutagens. Analysis of the metabolites of B[a]P to understand which of the many mutagenic metabolites may be responsible for the large deletions showed clearly that the "anti" 7,8-diol-9,10-epoxide was the major inducer of large lesions (more than 60% were large deletions compared to 38% for B[a]P itself). The effect of intact *uvrB* repair was examined with both B[a]P and IQ in strain TA1978. B[a]P showed no CG deletions. Even more remarkable was the IQ response which showed 42% large deletions and insertions compared to 0% with TA1538. The effect of error-prone repair (pKM101 plasmid) on B[a]P mutations (TA98) was similar to TA1538 with the exception of 4 unique lesions each showing a deletion and insertion at the same mutation site. (Work performed under auspices of the USDOE by the Lawrence Livermore National Laboratory under contract W-7405-ENG-48 and supported by IAG 222-01-ES-10063 between NIEHS and DOE.)

D 111 REACTIVITY OF FECAPENTAENE-12 TOWARDS THIOLS, DNA AND THESE CONSTITUENTS IN CULTURED HUMAN CELLS, R.C. Grafström, J.M. Dypbukt, C.C. Edman, T. Kakefuda* and C.C. Harris*, Karolinska Institutet, S-104 01 Stockholm, Sweden and National Cancer Institute*, Bethesda, MD 20892.

Micromolar concentrations of fecapentaene-12, a mutagen found in human feces, decrease survival measured as colony-forming efficiency and membrane integrity of cultured human fibroblasts. Fecapentaene-12 also decreases the content of cellular free low-molecular-weight thiols including glutathione. Fecapentaene-12 reacts directly with glutathione by causing both decreased levels of free thiol and some concomitant formation of oxidized glutathione indicating that thiol depletion is a result of both alkylation and oxidative reactions. Exposure of cells to 2 or 5 μM fecapentaene-12 causes significant amounts of DNA-interstrand cross-links and DNA-single strand breaks, respectively, whereas exposure to a higher concentration of fecapentaene-12, i.e. 10 μM also causes significant DNA-protein cross-links. Moreover, fecapentaene-12-induced DNA single-strand breaks accumulate in the presence of DNA polymerase inhibitors. The reaction of fecapentaene-12 with isolated plasmid DNA also results in primarily interstrand cross-links and strand breaks. Taken together, these studies show that fecapentaene-12 is a potent genotoxic agent that can react with cellular thiols and cause several types of DNA damage that also partly involve the DNA excision repair pathway.

D 112 TIME FRAME OF ACTIVATION OF ALTERNATIVE SITES OF REPLICON INITIATION IN RODENT CELLS, T. Daniel Griffiths and Su Y. Ling, Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115

For several years we have been examining how mammalian cells are able to completely replicate their DNA despite the presence of mutagenic/carcinogenic lesions that block DNA fork progression. Recently, using DNA fiber autoradiography, we published data (Mutat. Res. 184, 39-46, 1987) indicating that exposure of wild type (AA8) as well as excision deficient Chinese hamster ovary cells (UV-5 CHO) to UV light results in the use or activation of alternative sites of replicon initiation. This allows cells to replicate sections of DNA that do not contain a "normal" site of initiation yet contain lesions that block replication from either direction. In order to determine how rapidly these sites are activated, we altered our high/low specific activity labeling protocol. We determined that in order to detect post-pulse figures, the minimum length of labeling in high specific activity ^{3}H-thymidine was 10 min. Even with this 10 min high/35 min low specific activity labeling protocol, activation of alternative sites of replicon initiation was evident in both CHO AA8 and UV-5 cells immediately after exposure to 5.0 J/m^{2}. Thus, this activation is apparent before there is substantial blockage of replication, suggesting that other factors such as lesion-induced distortion of DNA may trigger this activation of alternative sites of replicon initiation. This work was supported by U.S.P.H.S. grant CA 32579.

D 113 A DOMAIN OF HYPERMETHYLATION PRESENT IN THE C-H-*RAS* REGION IN ADENOCARCINOMAS OF THE BREAST AND LUNG, Terrence J.Hall, Lori Jardines, David Baker, J.David Beatty, Jose J.Terz and Steven S. Smith, Division of Surgery, City of Hope, Duarte, CA 91010

We have compared the DNA methylation patterns in the regions of the c-H-*ras* gene near codon 12 and 3' VNTR sequence in adenocarcinomas and adjacent tissue. The 602 bp *Sma*I fragment of plasmid pT24-C3 containing the activated codon 12 sequence and the 987 bp *Msp*I fragment containing the 3' VNTR region were subcloned, and ^{32}P labelled. Genomic DNA from adenocarcinomas and adjacent tissue was digested to completion with either *Hpa*II or *Msp*I restriction endonucleases. Southern blots were probed serially with the "VNTR" probe and the "codon 12" probe. Our results demonstrated *increased* levels of methylation at numerous CCGG sites surrounding the VNTR region in adenocarcinomas of breast (5 specimens) and lung (4 specimens) compared to adjacent tissue. Heavy methylation of CCGG sites in the region surrounding codon 12 was found in *both* tumor and adjacent tissue. This suggests that extensive methylation in the vicinity of the codon 12 activation site *expands* to include the region near the VNTR during tumorigenesis. This *expansion* of the hypermethylated domain may identify important events associated with initiation or progression in human cancer.

D 114 MELANIN IS A PHOTO- AND RADIOSENSITIZER FOR OXIDATIVE DAMAGE IN DNA OF CLOUDMAN S91 MELANOTIC MELANOMA CELLS AND IN DNA *IN VITRO*, Helene Z. Hill, Karen Hubbard-Smith, Christine A. Huselton and George J. Hill, *Departments* of Radiology and Surgery, New Jersey Medical School, Newark, NJ 07103

The fact that individuals with black skin rarely get skin cancer is attributed to melanin in their epidermis. Melanin can protect the skin from solar damage by scattering and absorption. However, the pigment itself is very photoreactive and, when illuminated, takes up oxygen, emits superoxide, hydrogen peroxide and hydroxyl radicals. Isogenic melanotic and hypomelanotic Cloudman S91 mouse melanoma cells were irradiated with UVC. The DNA was isolated, purified, hydrolyzed to bases and analyzed by HPLC. The hydrolysis method allows for complete recovery of thymine glycol (TG) as TG and its derivative, hydroxymethyl hydantoin. TG was positively identified in the hydrolysate from UVC-irradiated melanotic melanoma cells by gas chromatography - mass spectroscopy, but was not present in similarly irradiated EMT6 mouse mammary carcinoma cells. The extent of oxidative damage observed in DNA was a function of UVC dose and intracellular melanin concentration. Hypomelanotic cells had small, but measurable oxidative damage. In vitro, DOPA-melanin (DM) induces sites in calf thymus DNA that are recognized by an antibody to TG and by endonuclease III. Furthermore, TG induction in DNA by ionizing radiation is enhanced by the presence of DM during irradiation. These findings indicate that melanin can act as a photo- and radiosensitizer. They have important implications regarding the etiology of skin cancer, particularly melanoma.

D 115 ACQUISITION OF RESPONSIVENESS TO CHEMICAL CARCINOGEN BY RAT EMBRYO FIBROBLASTS EXPRESSING AN EXOGENOUS C-MYC GENE. W.-L. WENDY HSIAO, RONNIE HANECAK, MARK CASTRO AND HUNG FAN, DEPT. OF MOL. BIOL. AND BIOCHEM., UNIV. OF CALIFORNIA, IRVINE, CA92717
In the current studies, two retroviral vectors, namely ch#3 which expresses c-myc gene from wild-type Murine Leukemia Virus (MuLV) LTR and ch#4 which expresses c-myc gene driven by a hormone-inducible MMTV-MuLV hybrid LTR (Hanecak et.al., unpublished deta) were employed. Each vector, also containing the neomycin gene, was introduced to Rat 6 cells seperately and a series of G418 resistant colonies were isolated. Clones containing ch#3 sequences display various degrees of altered morphology. They form orderly, but densily packed cells, grow to higher saturation density and yield small colonies in soft agar. Transient exposure of the ch#3-clones to benzo(a)pyrene(BP) induced the formation of distinct, large colonies in soft agar, whereas the parental Rat 6 cells remained as single cells in soft agar whether they were treated with BP or not. In a seperate experiment, we obtained two neo+ clones upon infection of ch#4 in Rat 6 cells. These clones closely resemble the normal Rat 6 cells in the absence of dexamethasone. Addition of dexamethasone altered the cell morphology and enhanced growth in soft agar. We also found that the responsiveness to BP is dexamethasone dependent. Thus, the acquisition of the sensitivity to BP of the c-myc containing Rat 6 cells is directly associated with the expression of c-myc sequences. These c-myc containing cells may be employed as a model system to study interation between oncogenes and chemical carcinogens in the process of multistage carcinogenesis. Supported by NIH-ES-86-16.

D 116 DNA DAMAGE AND ALTERED THYMIDYLATE SYNTHESIS IN LYMPHOCYTES FROM METHYL DONOR DEFICIENT RATS, S.J. James and L. Yin, Division of Nutritional Sciences, School of Public Health, UCLA, and GRECC, VAMC West Los Angeles, Los Angeles, CA 90024
Tumor induction with chronic feeding of methyl donor deficient diets is well-established; however, the biochemical and molecular mechanisms which predispose to tumorigenesis in this model are not well understood. In vitro studies have indicated that "thymidylate stress," secondary to a decrease in folate-dependent thymidylate synthesis, promotes certain cancer-associated aberrations in DNA structure and nucleotide metabolism. Given the metabolic interdependence between methionine, choline and folic acid, the purpose of the present investigation was to examine DNA damage and pathways of thymidylate synthesis in lymphocytes from Fischer 344 rats fed one of four semi-purified diets: 1) low in methionine, lacking in choline; 2) low in methionine, lacking in choline and folic acid; 3) lacking in folic acid only; or 4) a supplemented control diet. The results indicated that a decrease in thymidylate synthesis is induced in mitogen-stimulated lymphocytes from methionine/choline deficient rats independent of folate levels in the diet. This biochemical alteration was associated with a significant increase in DNA strand break accumulation and a decrease in the DNA repair-associated pteridine nucleotide, NAD. These changes were consistently exaggerated by superimposed folate deficiency. A synergistic lipotropic interaction between folate deficiency and methionine/choline deficiency was additionally observed in the liver. Taken together, the results suggest that folate deficiency interacts with methionine/choline deficiency to potentiate symptoms of methyl donor depletion and that alterations in folate metabolism may be related to DNA damage and mechanisms of carcinogenesis in this model.

D 117 **THE HUMAN HYPERVARIABLE REGION CONSENSUS IS AT THE BREAKPOINTS OF ABERRANTLY-REARRANGED c-myc, bcl1, bcl2 and tcl2.** **T.G. Krontiris, A.M. Krowczynska and R.A. Rudders, Departments of Medicine and Molecular Biology and Microbiology, Tufts-New England Medical Center, Boston, MA 02111.** **The genesis of hypervariable regions (minisatellites, VTRs), as well as the mechanism(s) by which they become polymorphic, are poorly understood. Characterization of our own VTR isolates and subsequent comparison with published VTRs has allowed us to refine the previously-defined consensus. Given the strong similarity between our consensus (GC[A/T]GG[A/T]GG) and the procaryotic activator of recombination, chi (GCTGGTGG), we actively sought evidence for the presence of the VTR consensus at known recombination events. First, we observed a 7/8 match of this consensus precisely at the crossover between the human immunoglobulin genes $\gamma\psi$ and $\gamma 1$ in the recombination event which created the immunoglobulin gene $\gamma 3$. We then asked if the VTR consensus was associated with somatic recombination in lymphocytes, particularly the aberrant immunoglobulin rearrangements involving the oncogenes, c-myc, bcl1, bcl2 and tcl2. Four 8/8 matches (c-myc and bcl2) and five 7/8 matches (c-myc, bcl1 and tcl2) of the consensus occurred with 12 bp of the breakpoint in 24 tumors. The probability of this observation by chance was 8×10^{-11}. The bcl2 consensus marked the 5' border of the major breakpoint cluster of this oncogene; two independent rearrangements occurred within 2 bp of the sequence and another five within 100 bp. This result suggests that the VTR consensus is employed as somatic recombination signal in lymphocytes.**

D 118 EVIDENCE THAT THE PRESENCE OF AN IMMORTAL DNA STRAND IN STEM CELLS IS UNLIKELY. Toshio Kuroki and Yoshinori Murakami*, Department of Cancer Cell Research, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108, and *Present address: National of Cancer Center Research Institute, Tsukiji, Chuo-ku, Tokyo 104, Japan.

According to the hypothesis proposed by Cairns (Nature, 255, 197-200, 1975), stem cells retain the older of the two parental DNA strands whereas differentiating daughter cells receive the newly synthesized strand, thus a set of the "immortal strand" persisting in stem cells through successive cell divisions. Stem cells could be protected in this manner against errors arising during DNA replication. To test this hypothesis, five successive divisions were induced in basal epidermal cells *in vivo* by two injections of cholera toxin into mouse skin and labeled cells by ^{3}H-thymidine at the first cell cycle were chased for 50 days. If the selective segregation occurs, the labeled strand should be transferred into a non-stem daughter cells and no labeled mitotic cells are seen after the third cell cycle. Three lines of evidence suggest that the immortal strand hypothesis is unlikely; (1) persistent labeling of epidermal basal cells for 50 days; (2) persistent labeling of mitotic cells of the epidermis over five cell cycles; and (3) logarithmic decrease of grains in mitotic cells through cell divisions.

D 119 **ENHANCEMENT OF MHC CLASS I PROTEIN SYNTHESIS BY DNA DAMAGE IN CULTURED HUMAN FIBROBLASTS AND KERATINOCYTES**, Michael E. Lambert[1], Zeev A. Ronai[2], I.B. Weinstein[2], and James I. Garrels[3], [1]Dept. of Immunology, Research Institute of Scripps Clinic, La Jolla, Ca. [2]Comprehensive Cancer Center, Columbia University, New York, NY. [3]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. Exposure of primary human fibroblasts or SV40 transformed human keratinocytes (SVK14) to several different classes of DNA damage, including Ultraviolet Light C (254 nm) (UVC), resulted in a rapid increase in the expression of Human Major Histocompatibility Class I (MHC-I) protein synthesis. MHC-I induction was also detected after exposure to low doses of the protein synthesis inhibitor, cycloheximide, suggesting that MHC-I induction by DNA damage may be a component in a derepressible cellular "SOS" pathway. UVC induced MHC-I expression was also detected in quiescent cultures of human fibroblasts indicating that the increase in synthetic rate was not a secondary consequence of DNA damage induced arrest of DNA synthesis. Enhancement of MHC-I protein synthesis was more pronounced in fibroblast strains derived from patients with Xeroderma Pigmentosum, suggesting a correlation between the persistence of unrepaired DNA damage and the extent of induction. The enhancement in protein synthesis was accompanied by an increase in both steady state levels of MHC-I specific mRNA and by an increase in cell surface expression of MHC-I molecules.

D 120 TOPOLOGICAL CHANGES OF MINICHROMOSOMES DURING DNA REPAIR IN FROG OOCYTES, Randy J. Legerski, Joseph Penkala, Carolyn Peterson and David Wright, Department of Molecular Genetics, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030. In previous work we have shown that excision repair of UV-induced damage is efficiently removed upon injection of plasmid DNA into Xenopus oocytes. Others have shown that plasmid DNA injected into oocytes is assembled into minichromosomes. We are investigating the topological changes that occur to minichromosomes during the course of excision repair of UV dimers. Our observations indicate that there is a decrease in negative superhelicity during the initial stage of repair and then a slow return to the original, unperturbed distribution of topoisomers. We are currently interpreting these results to indicate that nucleosome unfolding occurs during the early stages of repair to allow access to underlying lesions followed by a reformation of chromatin structure. Experiments are in process to test this hypothesis.

D 121 REVERSE MUTATIONS INDUCED IN AN ENDOGENOUS DEFECTIVE MuLV p15^{gag}: A SENSITIVE IN VIVO ASSAY FOR CHEMICAL MUTAGENESIS. John A. Mercer, Kenneth H. Lee, Bjorn Nexø[#], Nancy A. Jenkins, and Neal G. Copeland, BRI-Basic Research Program, NCI-FCRF, Frederick, MD; [#]Novo Research Institute, Bagsvard, Denmark. DBA/2 mice carry a single endogenous ecotropic murine leukemia provirus, Emv-3, which is expressed in only one-third of adult mice, suggesting that it is replication defective. Previous experiments have localized this defect to a single nucleotide substitution in codon 3 of p15^{gag}, which results in the substitution of proline for glutamine and thus inhibits myristylation of Pr65^{gag}. Since this myristylation is required for virus assembly, expression of Emv-3 is effectively halted. Percutaneous treatment of DBA/2 mice with the carcinogen and mutagen 7,12-dimethylbenzanthracene (DMBA) rapidly induces ecotropic virus expression in approximately 95% of treated mice. We hypothesized that this induction is the result of a reverse mutation in codon 3 of p15^{gag}, leading to amino acid substitutions which allow for myristylation. We have tested this hypothesis by isolating ecotropic viruses from DMBA-treated mice, amplifying p15^{gag} coding sequences by the polymerase chain reaction, and determining the DNA sequence of this region. In all of the viruses examined, single nucleotide substitutions were found in codon 3 of p15^{gag}, leading to pro-->ser and pro-->gln substitutions which restore the myristylation site required for virus assembly. This system provides valuable information on the specificity of mutations induced by DMBA and other mutagens in higher eukaryotes. More importantly, this system provides an extremely sensitive in vivo assay for mutagenic activity, since one mutation in one cell can result in viremia.

D 122 TRANSCRIPTION TERMINATING LESIONS INDUCED BY BIFUNCTIONAL ALKYLATING AGENTS IN VITRO. Russell O. Pieper, Bernard W. Futscher, and Leonard C. Erickson. Section of Hematology/Oncology, Loyola University of Chicago, Maywood, IL 60153. Nitrogen mustard (HN2), C2 (an active derivative of cyclophosphamide), chlorambucil (CBC), and melphalan (L-PAM) are bifunctional DNA alkylating agents. The present study was initiated to determine if DNA damage induced by these agents inhibits transcription in vitro, and to determine if sites of transcription termination correlate with N-7 guanine alkylation caused by these agents. For in vitro transcription studies, linearized plasmid pGEM-3Z containing the 413 bp Pst fragment of human c-myc exon 2 was incubated with biologically relevant concentrations of the drugs for one hr at 37°C, precipitated, and transcribed using bacteriophage SP6 or T7 RNA polymerase and ^{32}P-UTP. The RNA products were analyzed by PAGE and autoradiography. Sites of guanine N-7 alkylation were determined by a modified Maxam-Gilbert DNA sequencing technique. At the exposures used, sequencing studies showed that template molecules could be alkylated at any guanine. This alkylated DNA, however, when transcribed resulted in RNA molecules truncated not at every alkylated guanine, but at various discrete sites throughout the template. Transcription of DNA incubated with HN2 terminated (8 of 10 sites examined) at selected GG sites in the DNA template. Transcription of DNA exposed to activated C2 was unaffected. Transcription of DNA exposed to CBC terminated (13 of 14 sites examined) at selected GA or AG sites in the DNA template. Transcription of the DNA exposed to L-PAM terminated at AA sites in the template. These results suggest that DNA damage induced by some bifunctional alkylating agents can terminate transcription in vitro, and that the terminating lesions are not exclusively N-7 guanine adducts.

D 123 SPECIFIC CHROMOSOMAL INTEGRATION SITE OF H-RAS IN TRANSFORMED RAT EMBRYO CELLS, W. Gillies McKenna[§], Ken Nakahara*, Pat Eagle[†], Martha Sack[†] and Ruth J. Muschel[†], Departments of [†]Pathology and Lab. Medicine and [§]Radiation Oncology, University of Pennsylvania School of Medicine, Philadelphia, PA. and *NCI-Navy Medical Oncology Branch, Bethesda, MD

The H-ras oncogene transforms primary rodent cells at low frequency unless cotransfected with a cooperating oncogene such as v-myc. Furthermore, H-ras can be introduced alone into cells, be expressed and yet not induce transformation. These observations indicate that other changes must occur in a cell in addition to expression of activated ras for transformation to occur. Since most transformed cells show karyotypic alterations we questioned whether a common karytyopic alteration might accompany H-ras transformation. We undertook an analysis of seven independently derived clones of rat embryo cells transformed by human (T24) H-ras plus v-myc. All seven lines were evaluated by in situ hybridization using human ras, which does not cross-hybridize with the native rat gene, as a probe. In all seven lines a common site of integration for ras on the q arm of rat chromosome 3 (3q12) was found, although other sites were also noted in some lines. In four of the lines integration of ras was accompanied by deletion of the p arm of chromosome 3 or its possible translocation to chromosome 12. These findings suggest two possibilities which may not be mutually exclusive; one, that this site may be particularly receptive for integration and second, that integration of H-ras at this site might have an effect on an adjacent gene through insertional mutagenesis. Integration of H-ras on chromosome 3 or the non-random karyotypic changes that accompany ras integration at this site may be one of the other cellular changes which accompanies ras transfection to produce full transformation.

D 124 Genetic Hypervariability and Selection of Neoplastic Variants as a Mechanism of Transformation for SV40 Large T Protein F. Andrew Ray[1,2], Janet L. Cooper[1], David S. Peabody[2], L. Scott Cram[1], Paul M. Kraemer[1] [1]-Cell Biology Group, MS-M888 Los Alamos National Laboratory, Los Alamos, N.M. 87545 [2]-Dept. of Cell Biology, School of Medicine, University of New Mexico, Albuquerque, N.M. 87131
To define the role of SV40 large T antigen in the transformation and immortalization of human cells, we have constructed a plasmid lacking most of the unique coding sequences of small t antigen. The promoter for T antigen which lies within the origin of replication was deleted and replaced by the Rous sarcoma virus promoter. This minimal construct was co-electroporated into normal human fibroblasts of neonatal origin along with a plasmid containing the neomycin resistance gene(neo). Three G418 resistant, T antigen positive clones were expanded and compared to three T+ clones having received the pSV3neo plasmid (capable of expressing large and small T proteins and having two SV40 origins of replication). Immediately after clonal expansion, several parameters of neoplastic transformation were assayed. Low percentages of T antigen positive populations were anchorage independent or capable of forming colonies in 1% fetal bovine serum. Large numbers of dead cells were continually generated in all T+ populations. Frequent numerical and structural chromosomal aberrations were observed. Control cells that expressed the neo gene but not the T protein did not exhibit these karyotype changes. We suggest that the role of T protein in the transformation process is to generate genetic hypervariability leading to various consequences including transformation and cell death.

D 125 EFFECT OF DIFLUORO-METHYLORNITHINE (DFMO), A POTENTIAL ANTICANCER AGENT, ON MUTATION AT THE THYMIDINE KINASE LOCUS IN L5178Y ($tk^{+/-}$) MOUSE LYMPHOMA CELLS, Colette J. Rudd, SRI International, Menlo Park, CA 94025. DFMO is a promising anticancer agent because it is capable of inhibiting tumor growth in vivo. It is an irreversible inhibitor of ornithine decarboxylase, the rate-limiting enzyme for polyamine synthesis. The effects of this chemical on mutation at the thymidine kinase locus have been studied using a modified mutagenesis assay in which cells are suspended in semisolid medium during the expression and selection periods. This technique prevented changes in the ratio of mutant to wildtype cells during the two-day period required for expression of new mutations. Mutant cells were selected by allowing trifluorothymidine (TFT) to diffuse through the semisolid medium after the expression period. The cultures were incubated an additional 10 days for mutant colony formation. Cells were pretreated with 0.25 to 10 mM DFMO for two days prior to determining the mutation frequency in the cell populations. This treatment depleted cellular polyamines and inhibited cell growth. Treated cells were still viable, as the cloning efficiency of the cells was comparable after removal of DFMO. The frequency of TFT-resistant colonies in the cultures was substantially reduced in cultures pretreated with DFMO compared to untreated cultures, suggesting that fewer spontaneous mutations occurred as a result of the DFMO treatment.

D 126 A MODEL SYSTEM FOR STUDYING INDUCED DNA REARRANGEMENTS. Schnipper LE*, Sedivy JS†, Jat P†, Chan V*, Sharp PA†. Beth Israel Hospital, Harvard Medical School, Boston, MA*, Center for Cancer Research, MIT, Cambridge, MA†.

The molecular basis for DNA rearrangements that may contribute to the initiation of cancer, and of the genetic instability that is associated with tumor progression, is unknown. An experimental model with which to study this problem has been developed by infecting NIH 3T3 cells with the retroviral vector ZIPNeoSV(X)1, that has been modified by deleting the viral enhancer which is contained within a 100 bp segment in the long terminal repeat (LTR) sequences. Whereas the wild type viral vector expresses neo, and confers resistance to genectin sulfate (G418), cells containing an integrated copy of the enhancer-deleted construct, so called NIH/EN cells, are G418 sensitive.

The NIH/EN model system has been employed to determine the ability of selected stimuli to induce DNA rearrangements that are associated with activation of neo, and G418 resistance. Whereas the spontaneous frequency of $G418^r$ in NIH/EN cells is $<5 \times 10^{-7}$, exposure either to the tumor promoting agent, TPA, UV irradiation plus TPA, or SV40 T antigen following transfection of NIH/EN cells with a temperature sensitive mutant of SV40-T, results in the emergence of $G418^r$ clones at frequencies of 5, 67 and $52/10^6$ cells, respectively. Analysis of selected $G418^r$ cell lines, including cloning of fragments containing rearrangements in the region of neo, has been employed to understand the basis of neo activation following exposure to SV40 T or TPA. These studies validate the use of the NIH/EN cells as a model system with which to study the induction of DNA rearrangements resulting in gene activation.

D 127 MODULATION OF EPITHELIAL CELL SENSITIVITY TO ASBESTOS FIBERS BY TRANSFORMING GROWTH FACTOR β. Ann Sesko & Brooke Mossman, Department of Pathology, University of Vermont College of Medicine, Burlington, VT 05405. A number of proliferative and cytotoxic responses similar to those induced by soluble tumor promoting phorbol esters occur in Hamster Tracheal Epithelial (HTE) cells exposed to asbestiform minerals. Cloned diploid HTE cells were examined in a number of short and long term biological assays for responsiveness to crocidolite asbestos in comparison to other fibers (chrysotile asbestos, Code 100 fiberglass) and particulates (riebeckite, antigorite, glass beads). Additionally, the influence of fetal bovine serum (FBS) on these effects were assessed. In 10% FBS, 1 ug/cm^2 dish of crocidolite stimulated [3H]thymidine incorporation, and concentrations >5 ug/cm^2 dish were cytotoxic in an assay of colony forming efficiency (CFE). In 2% FBS, a ten-fold lower concentration of crocidolite caused increases in [3H]thymidine and higher concentrations were required for cytotoxic responses. The inhibition of crocidolite-induced proliferative responses could be achieved by supplementing 2% FBS medium with 3 ng/ml transforming growth factor β (TGF-β). At this concentration of TGF-β, HTE cells: 1) morphologically resemble fibroblastic cells; 2) do not exhibit crocidolite-stimulated proliferation in the CFE assay; and 3) demonstrate complete inhibition of crocidolite-induced elevations in [3H]thymidine incorporation. Results suggest that factors present in serum such as TGF-B strongly influence the sensitivity of HTE cells to crocidolite asbestos. Thus, manipulation of cell culture conditions may reveal proliferative responses to toxic and carcinogenic agents. Supported by grants #T32CA09286 and #RO13350106 from the NCI.

D 128 IDENTIFICATION OF MULTIPLE DNA REPAIR GENES ON HUMAN CHROMOSOME 19 ATTRIBUTABLE TO HEMIZYGOSITY OF A HOMOLOGOUS REGION IN THE CHO GENOME, M.J. Siciliano[1], L.L. Bachinski[1], L.H. Thompson[2], C. Weber[2] and R.L. Stallings[3], [1]The University of Texas M.D. Anderson Cancer Center, Houston, Tx., [2]Lawrence Livermore National Laboratory, Livermore, Ca., [3]Los Alamos National Laboratory, Los Alamos, NM.
Recessive DNA repair mutants representing different complementation groups have been isolated from Chinese hamster ovary (CHO) cells and the human genes that correct those repair deficiencies, named either Excision or X-ray Repair Cross Complementing (ERCC or XRCC) have been identified and chromosomally assigned by somatic cell hybridization of human cells with a CHO mutant. The assignments of 4 different repair genes have been published. Two of these genes, ERCC1 and XRCC1, map onto human chromosome 19. Here we report by two methods, identification of the correcting human chromosome in hybrids made with CHO excision repair mutant from complementation group 2 and by hybrididization of the cloned human repair gene to Southern blots of a hybrid clone panel informative for human chromosomes, the assignment of a third repair gene, ERCC2 to human chromosome 19. Since certain human chromosome 19 isozyme markers have had their homologous genes mapped onto the hamster chromosome 9 and that chromosome had been shown to be hemizygous in CHO cells, cDNAs of the three human chromosome 19 repair genes were hybridized to blots of somatic cell hybrids informative for Chinese hamster genes. All three segregated concordantly with Chinese hamster chromosome 9. These data suggest that many more rodent DNA repair mutants, and therefore many more human DNA repair genes, may be identified by using rodent cell lines hemizygous for regions homologous to different regions of the human genome. (Support from CA34936 and DOE).

D 129 CpG IS A DRAMATIC HOTSPOT OF GERMLINE MUTATION, Steve S. Sommer, Dwight D. Koeberl, Jean-Marie Buerstedde, and Cynthia D.K. Bottema, Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, MN 55905. Genomic amplification with transcript sequencing (GAWTS) is a three-step procedure that allows direct genomic sequencing. More than 100,000 bp of sequence was generated by GAWTS from eight regions of the factor IX gene which include the putative promoter region, the coding region, and the splice junctions. The sequence of all eight regions was obtained from 20 unrelated normal individuals of defined ethnicity and subsequently from 21 hemophiliacs in different families. Three major conclusions emerge: (1) The rate of polymorphism in these eight regions of functional significance has been measured in an X-chromosomal gene and it is about 1/3 of the average rate observed for intronic and intergenic sequences on the X chromosome. The rate is low enough that the causative mutation should be the only sequence change seen in the overwhelming majority of hemophiliacs. (2) In the observed mutations, the rate of transitions at CpG is elevated by an estimated 62-fold, presumably due to lack of repair of thymidine generated by the spontaneous deamination of 5-methylcytidine. The dramatic enhancement of mutation at CpG suggests that a significant minority of germline mutations in humans may well be due to "an endogenous system" which is independent of environmental mutagens. Such a system may also produce some of the somatic mutations which underlie neoplasia. (3) High quality, reproducible sequence data can be obtained on a time scale that makes direct carrier testing and prenatal diagnosis feasible.

D 130 MUTATION IN CHINESE HAMSTER CELLS CAN BE DELAYED AT LEAST 9-CELL DIVISIONS AFTER UV-LIGHT TREATMENT, Thomas Stamato*, Nicholas Denko, Amato Giaccia, Renate MacLaren and Edwin Richardson, The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, PA 19104. The ability of ultraviolet light to induce delayed mutational events at the glucose-6-phosphate dehydrogenase (G6PD) locus was examined by treating single Chinese hamster ovary (CHO) cells with UV-light, isolating single clones, replating, and staining for glucose-6-phosphate dehydrogenase activity. Four out of 930 clones produced G6PD-negative staining colonies in the ratios expected for mutations occurring at the 7th cell division (128 wild-type clones to 1 mutant), 8th division (256:1), 9th division (512:1), and before the first cell division (all mutants). In control experiments, no G6PD-negative staining colonies were observed when 947 untreated colonies were examined for mutation. These are stable mutants with no detectable G6PD activity in cell extracts. Thus UV-light, like ethyl methane sulfonate (Somat. & Mol. Gen. 13:57, 1987), produces mutational events which can be delayed for at least 9 cell generations after the original mutagen treatment. In contrast, in yeast and bacteria mutations occur within the first or second cell division after mutagen treatment. The appearance of delayed mutational events in mammalian cells suggests that there are fundamental differences in the mutagenic processes of procaryotic organisms and mammalian cells. This phenomenon could well be at least a partial explanation for the long delay between exposure to a carcinogen and the development of observable tumors in the animal.

D 131 THE MECHANISM OF DNA REPLICATION IN HUMAN LEUKEMIC CELLS AND ITS DISRUPTION BY ALKYLATING AGENTS AND CYTOTOXIC DRUGS,
Bernard W. Stewart, Daniel R. Catchpoole and Maria Kavallaris, Children's Leukaemia and Cancer Research Unit, Prince of Wales Children's Hospital, Randwick, Sydney, NSW 2031, Australia.
Incorporation of [^{3}H]thymidine into DNA of randomly dividing or synchronized leukemia cells (CCRF-CEM) was monitored by elution of deproteinized preparations from benzoylated-DEAE-cellulose using NaCl and caffeine solutions. After periods from 30 seconds to 10 minutes, radioactivity recovered in caffeine solution by stepwise elution (DNA with single stranded regions) was sufficient for caffeine gradient elution. By this procedure, two classes of structural defect were detected. Some replicating DNA exhibited single-stranded regions of approximately 200 nucleotides whilst most radioactivity was associated with DNA containing single stranded regions from 900 to approximately 4,000 nucleotides. Agarose gel electophoresis indicated that both these single stranded regions were associated with polymerization of high molecular weight DNA. On the basis of current models, the two classes of single stranded regions in replicating DNA are presumptively associated with replicating forks and strand separation respectively, although direct sizing of such regions has not been previously possible. The single stranded regions were differentially affected by exposure of the cells to methyl methanesulfonate or specific inhibitors of DNA replication. The former affected progress of replicating forks whereas the latter modified single stranded regions associated with strand separation.

D 132 HOMOLOGOUS RECOMBINATION BETWEEN REPEATED CHROMOSOMAL CODING FOR HYGROMYCIN RESISTANCE SEQUENCES IN VARIOUS HUMAN CELL LINES WHICH DIFFER IN NUCLEOTIDE EXCISION REPAIR CAPACITY, Tohru Tsujimura, Veronica M. Maher, and J. Justin McCormick, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824-1316 U.S.A. We are studying the mechanism(s) of spontaneous and carcinogen-induced homologous recombination in human cells and investigating the role of DNA repair in these processes. For this purpose we have transfected a series of human cells with a plasmid carrying duplicated copies of the gene coding for hygromycin resistance. The plasmid, pTPSN, was generously provided by R. Michael Liskay, Yale University. Neither hygromycin gene can express a functional enzyme because each contains a HindIII restriction site inserted at a different location. However, a productive recombinational event between the two defective genes can lead to hygromycin resistant cells. The gene for neomycin resistance is located in the sequence between the two hygromycin genes. This allows us conveniently to select for transformants that have integrated the plasmid stably, and also permits us to readily determine whether the recombination event between the two hygromycin genes involves loss of the intervening sequences without the need for Southern blot analysis. This plasmid was transfected into a normally-repairing, infinite lifespan human cell line, KMST-6, and two nucleotide excision repair-deficient XP cell lines, XP20S from group A and XP2YO from group F, and the neomycin resistant transfectants were screened for those capable of undergoing productive recombination to yield hygromycin resistant colonies. Southern blot analysis of DNA from several cloned transfectants of each cell line indicated that the portion of the plasmid containing the two hygromycin genes and the intervening sequence was intact, and that several transfectants contained only one, or at most, 2 or 3 copies of the plasmid integrated into the genome. Fluctuation tests have determined that the spontaneous rates of recombination in these cell strains range from 2×10^{-6} cells per cell generation to 10×10^{-6} cells per cell generation. Studies to date on UV-induced recombination in these cell strains indicate that the frequency of recombination is higher in the XP cell strains than in the KMST-6 cells, indicating that nucleotide excision repair acts to prevent recombination. This research was supported by Contract 87-2 from the Health Effects Institute (HEI) and NIH/NCI CA21253.

D 134 SPECIFIC CHROMOSOME ALTERATIONS THAT CORRELATE WITH PROGRESSION TO IMMORTALITY IN RAT TRACHEAL EPITHELIAL CELLS TRANSFORMED WITH MNNG, Cheryl Walker, Mitsuo Oshimura, Paul Nettesheim and Sumiyo Endo, NIEHS and CIIT, Research Triangle Park, N.C. 27709. Primary rat tracheal epithelial cells can be transformed in vitro by MNNG. The earliest recognizable morphological transformant is the enhanced growth variant (EGV), characterized by enhanced proliferative capacity. Transformed EGV colonies can progress to give rise to immortal cell lines. The purpose of this study was to determine if specific chromosome changes occur which correlate with immortalization. A total of 34 EGV colonies were isolated, of which 5 were able to progress in culture to become immortal (≥ 100 pop. doublings). Cytogenetic analysis performed on passage 1 cells of the 5 immortalized cell lines indicated that non-random numerical and structural alterations were present in these cells. All 5 cell lines exhibited an additional copy of chromosomes 7,4, and 11 as a common or recurrent abnormality. These numerical alterations were rarely observed in the EGV colonies from which the cell lines were derived, suggesting that these alterations occurred during progression. Structural alterations involving chromosome 1 (resulting in a net gain of 1q) and chromosome 3 (3q) also occurred in 4 of 5 cell lines. In all cases, structural alterations involving 1q and/or 3q were detected in the primary EGV colonies from which the lines arose. Comparison of the frequency of the structural and numerical alterations in the immortal cell lines with their frequency in the 29 EGV colonies which did not become immortal indicated that these changes correlated ($p \leq .005$) with the ability to become immortal. These results suggest that structural alterations occur in primary EGV colonies which predispose cells to immortalization and that subsequent numerical changes occur during progression that correlate with acquisition of the immortal phenotype.

D 135 A NEW FORM OF AMPLIFIED DNA IN MAMMALIAN CELLS: PRECURSORS TO DMs, Brad Windle, Joe Ruiz, Dan Von Hoff*, Susan Carroll, Bruce Draper, and Geoff Wahl, Gene Expression Laboratory, The Salk Institute, La Jolla, CA 92037, and *Department of Medicine/Oncology, University of Texas Health Science Center, San Antonio, TX 78284.
Gene amplification of oncogenes and drug resistance genes in tumor cells has been associated with the progression of tumors and a cell's ability to survive treatment with chemotherapeutic agents. Amplified DNA has generally been observed to be manifested as double minute chromosomes (DMs) or homogeneously staining regions of chromosomes (HSRs). However, there have been observations of tumor cells with amplified oncogenes but no apparent DMs or HSRs. We have developed electrophoretic techniques for identifying submicroscopic extrachromosomal DNA elements (episomes) which range in size from 250 kb to 1400 kb in mammalian cell lines. These DNA structures represent a new form of amplified DNA and can constitute the majority of amplified DNA in a cell line. The following amplified genes have been found encoded in these episomes: c-myc, CAD, mdr (multi-drug resistance gene), and ada (adenosine deaminase). These episomes are maintained as supercoiled DNA molecules which replicate semi-conservatively and approximately once per cell cycle. Using the CAD episome as a model, we have shown the progression of episomes into multimers, eventually leading to DM formation. Since the largest of the episomes detected is the size of a small DM, it may be possible with these techniques to molecularly analyze DMs of any size. An important application of these techniques will be the characterization of amplified genes in tumor biopsies, since tumors containing DMs are much more prevalent than those containing HSRs.

D 136 TRANSCRIPTIONAL RESPONSES FOLLOWING EXPOSURE TO DIFFERENT QUALITIES OF RADIATION, Gayle E. Woloschak, Chin-Mei Liu, Carol A. Jones, Biological, Environmental, and Medical Research Division, Argonne National Laboratory, Argonne, IL 60439-4833
We examined the modulation of gene expression in Syrian hamster embryo (SHE) cells at various times following exposure to low doses of ionizing radiation. Early passage SHE cells were irradiated in plateau phase (>95% G_0/G_1 cells) with equitoxic doses of JANUS fission-spectrum neutrons (21 cGy), x-rays (75 cGy), or γ-rays (90 cGy), none of which induced more than 10% loss in cell viability. Nuclei and total cellular RNA were harvested at various times after exposure. Total transcription was decreased by 10-30% within the first 30 min. following exposure to all qualities of radiation, but the time for recovery to control values was markedly delayed in neutron-exposed cells (relative to similarly treated γ-ray or x-ray exposed cells). Since neutrons are three times more effective than γ-ray or x-rays at inducing chromosomal aberrations, mutations, and celullar responses, it is not surprising that total transcriptional responses to these different qualities of radiation are different. RNA preparations were also examined for levels of particular RNA species by Northern blot hybridizations. Levels of β-actin-specific RNA decreased within 15 min after exposure of the cells. Unlike transcription results, the kinetics of β-actin-mRNA repression were similar for all qualities of radiation (x-rays, γ-rays and neutrons) for 12 h post-irradiation. No alterations relative to untreated control cells in overall cell viability or the rate of cell-cycle progression were observed in cells either immediately or within 24 h postirradiation. (Work supported by the U.S. DOE, OHER, under contract No. W-31-109-ENG-38).

Genetic Mechanisms in Carcinogenesis and Tumor Progression

Growth Inhibitory Factors, Oncogenes and Tumor Suppression

D 200 THE DNA-BINDING ACTIVITY OF JUN IS INCREASED UPON ITS INTERACTION WITH FOS, Elizabeth A. Allegretto*, Tod R. Smeal*, Sadhana Agarwal+, Thomas Roberts+, Bruce M. Spiegelman+ and Michael Karin*, University of California, San Diego*; La Jolla, CA 92093 and Dana Farber Cancer Institute+; Harvard Medical School; Boston, MA 02115. The sequence specific transcription factor AP-1 is responsible for increased expression of a number of genes after treatment of mammalian cells with the phorbol ester tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA). TPA treatment leads to a rapid increase in the DNA-binding activity of AP-1. Recently, AP-1 was identified as the product of the c-JUN proto-oncogene. The viral oncogene v-JUN encodes a similar DNA-binding protein whose activity is essentially identical to AP-1. Jun/AP-1 and another proto-oncogene product, Fos, have been shown to interact with the promoter of an adipocyte specific gene. This interaction is due to formation of a tight heteromeric complex between Jun/AP-1 and Fos. While Jun/AP-1 is responsible for the specific binding of this complex to DNA, upon interaction with Fos, its transcriptional stimulatory activity is increased several fold. The interaction between Fos and Jun/AP-1 is important for the induction of specific target gene transcription by TPA, as this induction is prevented by expression of antisense FOS RNA. To further understand the mechanism of gene activation by TPA, the effect of Fos on the activity of Jun/AP-1 was examined. Upon interaction of Fos protein produced by a baculovirus vector with a trpE-vJun fusion protein expressed in E.coli, the DNA-binding activity of the fusion protein is increased several fold as determined by band shift assay. Furthermore, we show that Fos interacts with a truncated jun/AP-1 protein containing only the DNA-binding domain by immunoprecipitation with anti-fos and ant-jun antibodies.

D 201 Ras ONCOGENES IN HUMAN LUNG TUMORS ASSOCIATED WITH EXPOSURE TO CIGARETTE SMOKE, Steven H. Reynolds[1], Colleen K. Hunnicutt[1], Katie C. Brown[1], Ted Beattie[2], Ron Pero[2] and Marshall W. Anderson[1], National Institute of Environmental Health Sciences[1], Research Triangle Park, NC 27709; PMI-Strang Clinic and Kriser Lung Cancer Center[2], 55 East 34th Street, New York, NY 10016, The standard NIH/3T3 plate transfection technique, which selects exclusively for morphological transformation, and the NIH/3T3 transfection-nude mouse tumorigenicity assay, which depends only on tumorigenic potential, were both used to assay for potential oncogenes in human lung tumors. Seven of eight adenocarcinomas (AC) and two of three squamous cell carcinomas (SCC) were found to contain activated oncogenes by one or both of the assays used. Southern blot analysis identified the transforming gene as K-ras in four of the AC and H-ras in one of the SCC. The activating mutation in each of the K-ras genes was a GC to TA transversion in the first base of the 12th codon. A raf oncogene was detected in one of the AC. Three of the transforming genes did not hybridize with H-, K-, or N-ras, met, or raf. However Southern blot analysis with DNA from secondary nude mouse tumors derived from several primary nude mouse tumors exhibited consistent banding patterns with the human Alu probe. The Alu banding patterns suggest that the same oncogene was present in one AC and one SCC and a distinctly different gene was present in another AC. Thus, activated oncogenes were detected in a high percentage (9/11) of human lung tumors associated with cigarette smoking.

D 202 SIMULTANEOUS DETECTION OF ONCOGENE EXPRESSION AND CELL SURFACE MARKERS M.H.C. Bakkus, M.A. Versnel, H.J. Adriaansen, M.J. Evinger-Hodges and Th.W. van den Akker, Dept. of Cell Biology, Immunology and Genetics, Erasmus University, Rotterdam, The Netherlands.

The expression of several proto-oncogenes has been correlated with a number of malignancies. Using the RNA in situ hybridization (ISH) technique we were able to detect abnormal expression of these genes in small cell samples with a low percentage of tumor cells. This ISH technique may become a very useful diagnostic tool for early detection and monitoring of malignancies, especially when the cell-type expressing the oncogene can be further characterized by immunocytochemistry. We tried to combine these two techniques on the same cell sample by using biotinylated RNA probes for the ISH and monoclonal antibodies against cell surface determinants for further phenotyping of the cells. The biotinylated probe was detected with streptavidine-FITC. In a case of malignant mesothelioma we were able to detect c-sis expression in tumor cells from the pleural effusion. The malignant mesothelioma cells could be distinguished from the pleural effusions macrophages, which normally express the c-sis oncogene, by double-labeling with a TRITC-labeled monoclonal antibody against CD45. CD45 is a common leukocyte marker, present on macrophages but not on mesothelioma cells. The antibody labeling of the cells did not reduce the ISH signal. The combined methods enable us to determine more specifically the cell-type in which the mRNA molecule is expressed, which is of great importance for tumordiagnosis.
This investigation was supported by the Netherlands Cancer Foundation.

D 203 IN VITRO CHARACTERIZATION OF A MYELOMONOCYTIC CELL LINE DERIVED FROM HIGHLY LEUKEMIC BXH-2 RECOMBINANT INBRED MICE, Hendrick G. Bedigian, [1]Herbert C. Morse III, and R. Evans. The Jackson Laboratory, Bar Harbor, ME and [1]Laboratory of Immunopathology, National Institute of Allergy and Infectious Disease, Bethesda, MD. A factor independent cell line, designated bh2-1, was established from tumorous tissue of the recombinant inbred mouse strain, BXH-2. Phenotypic, biochemical, and molecular analysis of this cell line indicate that the cells are characteristic of hematopoietic cells of the myeloid lineage with very limited capacity for differentiation after stimulation with different lymphokines or phorbol ester. The bh2-1 cells clone efficiently in soft agar and are tumorigenic in syngeneic mice. Our results show that the bh2-1 cells release the growth factor CSF-1 but not GM-CSF, IL-3, IL-2, or interferon gamma. Northern analysis support these data and indicate that the absence of expression of GM-CSF, IL-3 and interferon, is at the level of transcription. In addition a low level of c-myc expression was detected in poly(A)-RNA prepared from bh2-1 cells but not to other proto-oncogenes assayed. Whether c-myc acts synergistically with CSF-1 to stimulate the proliferation of the tumorigenic bh2-1 cells in not known. However, preliminary evidence indicates that c-myc expression may be the results of cell passage and chromosomal changes and not the primary event in the transformation of these cells.

D 204 CHROMOSOME 17p ALLELE LOSS IN MULTIPLE COLORECTAL CARCINOMAS FROM A PATIENT WITH POLYPOSIS COLI. J.R. Coggins, T.M. Gomez, D.A. Ahlquist, R.B. Jenkins, H.A. Carpenter, and S.N. Thibodeau. Departments of Gastroenterology, Laboratory Medicine, Surgical Pathology, and Molecular Genetics, Mayo Clinic, Rochester, MN.
Loss of specific chromosomal loci may play a critical role in the development of embryonal and adult tumors. Recently, loss of chromosome 17p alleles has been found in 75% of sporadic colorectal cancers (NEJM 319:525-532, 1988). We describe the loss of chromosome 17p alleles in multiple colorectal cancers from a 24-year-old Caucasian female who appears to have acquired polyposis coli as a result of a new mutation. At colectomy, she had innumerable adenomatous colon polyps, two in situ cancers arising in adenomatous polyps, and four invasive colorectal cancers. Methods: High molecular weight DNA was extracted from peripheral WBCs, colonic mucosa, and cryostat sections of three adenomatous polyps and three invasive carcinomas. Sufficiently pure (70% or more tumor nuclei) cryostat sections could not be obtained from a fourth invasive cancer. The DNA was digested with Bam Hl, electrophoresed (0.8% agarose gel), transferred to a nylon membrane, and hybridized for 24 hours with the DNA probe pYNZ22 which had been labeled with 32p by random priming. Results: As compared with normal peripheral WBCs, loss of chromosome 17p alleles was seen in all three cancers from which adequate cryostat sections could be obtained, but not in normal mucosa or polyps. In two of the cancers, allele 2 was lost while in the remaining cancer, allele 1 was lost. Cytogenetic studies performed on two of the cancers were normal. Conclusion: In this patient, loss of chromosome 17p alleles may be associated with the development of colorectal carcinoma.

D 205 ISOLATION AND CHARACTERIZATION OF MUTATIONS AFFECTING THE RB1 GENE IN RETINOBLASTOMA TUMORS, James M. Dunn, Robert A. Phillips and Brenda L. Gallie, The Hospital for Sick Children Research Institute, 555 University Avenue, Toronto, Ont, Canada, M5G 1X8
In recent years the genetic basis of Retinoblastoma (RB), a rare heritable childhood cancer, has been elucidated and a candidate gene (4.7R) has been isolated. We have analysed the RNA isolated from 17 RB tumors by the RNase protection method and have detected 9 mutations of 4.7R which do not affect message length or abundance. This data suggests that point mutations play an important role in the inactivation of RB1. In addition germline mutations in bilateral patients have been detected. These data supports the genetic model which predicts that tumors isolated from RB patients carry mutations in both alleles of the RB1 gene and those patients with the heritable form of the disease carry a mutated RB1 gene in their germline. The polymerase chain reaction technique has been used to isolate and characterize these mutations. This pool of functional mutants affords an opportunity to study important regions and/or amino acid residues of this cancer suppressing gene.

D 206 HNE, A PRODUCT OF CELLULAR LIPID PEROXIDATION, MARKEDLY MODULATE CMYC EXPRESSION AND CELL DIFFERENTIATION, Farace M.G.[1,4], Fazio V. M.[2,4], Barrera G.[3], Martinotti S.[4], Frati L.[4], Manzari V.[4] and Dianzani M. U.[3], 1, Faculty of Medicine, University of Catanzaro, Italy, 2, Institute of Experimental Medicine, CNR, Rome, Italy, 3, Department of Medicine and Experimental Oncology, Univ. of Turin, Italy, 4, Department of Experimental Medicine, Univ. of Rome, viale Regina Elena 324, 00161 Rome, Italy.
The 4-hydroxy 2-3 trans nonenal (HNE) present a pronounced antiproliferative activity in tumor colture cells as well as in transplanted tumors. In order to investigate this effect we have studied the expression of genes related to proliferation, differentiation and transformation after HNE treatment. We found that HNE, at concentration very close to that constitutive in normal cells (approximately 1uM), specifically down modulates CMYC expression in K562 erytroleukemic cells, without exerting any toxic effect on cells. The molecular mechanism involved seems to be mainly post-transcriptional, as determined by run-off transcription assay. Moreover, after HNE treatment, expression of globin genes was enhanced 2-3 fold while housekeeping gene expression was unvariated. These results confirm the effect of HNE on tumor cell proliferation and suggest a specific role in cell differentiation.

D 207 **GROWTH-CONTROLLED EXPRESSION OF THE HUMAN ORNITHINE DECARBOXYLASE GENE.** Margaret A. Flanagan and Connie W. Woods, Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati, OH 45215
The increase in ornithine decarboxylase (ODC) activity observed in response to growth-stimulation is tightly controlled by transcriptional, translational and post-translational regulatory mechanisms. Measurements of steady-state ODC mRNA levels have been made under varied conditions in order to understand better the mechanism of pre-translational control. Northern blot analysis of total RNA isolated from serum-stimulated IMR90 cells (human diploid fibroblasts) showed that the proportion of ODC mRNA began to increase 60 min after stimulation and reached a plateau at 4 hr. Serum-stimulation for 4 hr in the presence of cycloheximide also induced increased ODC mRNA. Between 0.1 and 10 μg/ml cycloheximide the level of ODC mRNA was equal to serum-stimulation in the absence of drug, indicating that there is no requirement for de novo protein biosynthesis. This suggests that serum-stimulation activates a pre-existing positive regulatory protein. Superinduction of ODC mRNA was observed at 25 μg/ml cycloheximide and above. In addition, cycloheximide treatment of quiescent cells for 4 hr also resulted in an induction of ODC mRNA in a dose-dependent manner, although much less than in serum-stimulated cells. These data suggest the presence of a labile negative regulatory protein which disappears in the absence of protein biosynthesis. Taken together, the results of this study indicate that serum-stimulation activates a pre-existing regulatory protein, and that regulation of the ODC gene may be achieved by the interplay of this protein and other positive and negative regulatory proteins.

D 208 A (2'-5') A_n-DEPENDENT ENDORIBONUCLEASE: REGULATION OF ENZYME LEVELS BY INTERFERONS AND BY CELL GROWTH CONDITIONS, Georgia Floyd-Smith, Department of Zoology, Arizona State University, Tempe, AZ 85287

A (2'-5')A_n-Dependent Endoribonuclease (RNase L) is one enzyme mediator of interferon(IFN)'s anti-viral actions which may also mediate some of the cell growth inhibitory effects of IFNs. Levels of this enzyme have been determined in murine L cells, NIH 3T3 cells, m-mycl cells (NIH 3T3 cells transfected with a portion of the c-myc oncogene containing the 3'untranslated region) and in XH2 cells (NIH 3T3 cells transfected with a portion of the c-myc oncogene which lacks the 3'untranslated region). NIH 3T3 cells, XH2 cells and m-mycl cells all have similar levels of RNase L. L cells, however, may have 4 times higher levels of RNase L. Treatment of L cells at cell densities of 0.25 x 10^6 to 0.84 x 10^6 cells/ml with IFNαA/D, IFNβ or IFNγ results in a similar 2-4 fold induction of RNase L. IFN treatment of L cells at cell densities of 1.0-1.5 x 10^6 cells/ml does not result in an increase in the amount of RNase L as compared to controls. IFN treatment of NIH 3T3, m-mycl and XH2 cells results in a 2-3 fold increase in the levels of RNase L when cell densities are low, but does not induce RNase L when cell density is high. Prolonged maintenance of cells at high density is associated with a decrease in the rate of cell multiplication and in a drop in RNase L levels from a peak observed in cells initially approaching confluence. This decrease in RNase L is not accompanied by loss of cell viability suggesting that RNase L levels are down-regulated in post-confluent cells.

D 209 CEA AND CEA-RELATED RNA SEQUENCES IN PANCREATIC ADENOCARCINOMA, Marsha L. Frazier. Department of Medical Oncology. M.D. Anderson Cancer Center, Houston, Texas 77030.

Carcinoembryonic angtigen (CEA) and CEA-related sequences are coded for by members of a gene family. In blot analysis of RNA from the pancreatic adenocarcinoma cell line BxPC-3, three major bands and three minor bands of CEA homologous RNA are detectible. The three major bands co-migrate with three major bands from colon carcinoma and are 2.6 kb, 3.0 kb, and 3.5 kb in length. In colon, the 2.6 kb RNA codes for NCA, the 3.0 kb band codes for CEA. Recent studies demonstrate that CEA immunoreactivity is increased in certain cell lines in response to differentiating agent. We have studied the effects of the differentiating agent retinoic acid on BxPC-3. When cultured 5 days in the presence of 10^{-5}M, 10^{-6}M, or 10^{-7}M retinoic acid, growth was inhibited to a level of 82%, 67%, and 25% the level of untreated controls. Blot analysis indicates that of the three major CEA homologous RNAs detected, the most pronounced response was observed with the 2.6 kb RNA, which increased after 4 and 6 days of treatment with 10^{-6}M retinoic acid, or 6 days of treatment with 10^{-7}M retinoic acid. A very slight response was observed in the 3.0 kb RNA, while the 3.5 kb RNA band and the three minor bands remained unchanged. It is not clear if the mechanisms by which retinoic acid inhibits growth are related to those by which retinoic acid alters CEA and CEA-related gene expression. Whether induction of CEA homologous mRNA signals the entry of pancreatic tumor cells into a more differentiated state is currently being explored as well as the mechanism by which the expression is regulated by retinoic acid.

D 210 THE ROLE OF THE HUMAN RETINOBLASTOMA (Rb) GENE IN VARIOUS CANCERS, Yuen Kai Fung, Anne T'Ang, Koichiro Mihara, Wan-Rong Qiu, Kai-Jin Wu, Fu-Hui Zhang, Chin Du, Xiang-He Shi, A. Linn Murphree, Theresa Thompson, Yuan Yuan Chen, Childrens Hospital of Los Angeles, University of Southern California School of Medicine, Los Angeles, CA 90027

We have previously shown the involvement of the retinoblastoma gene in various human cancers including retinoblastoma[1], osteosarcoma[1], small cell lung carcinoma[2], breast tumor[3], fibrosarcoma and bladder carcinoma. Introduction of a chromosome 13 into an osteosarcoma cell line has led to the suppression of tumorigenicity of the cell line in nude mice[4]. Reintroduction of an intact Rb gene into a fibrosarcoma cell line led to altered morphology and retardation of growth. The cells became more elongated and the cell doubling time increased from 24 hours to 70+ hours. The results suggested that the Rb gene may have an effect on the growth of the tumor cell lines in vitro and in vivo.

References

1. Fung, et. al. Science 236 1657 (1987)
2. Yokota, et. al. Oncogene (1988) In Press
3. T'Ang, et. al. Science 242 263 (1988)
4. In Preparation

D 211 SUPPRESSION OF THE NEOPLASTIC PHENOTYPE OF A HUMAN LUNG CARCINOMA CELL LINE IN SOMATIC CELL HYBRIDS, **Edward W. Gabrielson, (Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD 21224), M. Edward Kaighn, and Curtis C. Harris (Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, MD 20892)**

Somatic cell hybrids have been constructed using the human lung carcinoma cell line HuT 292 and either normal human bronchial epithelial cells (NHBE) or "immortalized" but non-tumorigenic human bronchial epithelial cells (BEAS-2B). HuT 292 X NHBE hybrids senesced after 40-45 population doublings in culture, representing an extension of the 20-25 population doublings typically observed in NHBE cell cultures. In contrast, HuT 292 X BEAS-2B hybrids all appear to have indefinite doubling potential in culture, and have been further characterized for tumorigenicity in athymic nude mice. No tumors were observed for eighty percent of the HuT-292 X BEAS-2B hybrid clones, and the tumorigenic hybrid clones (twenty percent of clones isolated) had a 2-3 fold increase in the latency period before appearance of tumors when compared to the highly tumorigenic HuT 292. Thus, the indefinite life span and tumorigenic characteristics of the HuT 292 cell line are controlled by hybridization to normal or "immortalized" human bronchial epithelial cells. Such hybrid cell lines may be useful for identifying specific "tumor-suppressing genes" for the human bronchial epithelial cell type.

D 212 MODULATION OF INCIDENCE OF DIETHYLNITROSAMINE (DENA) INDUCED PRENEOPLASTIC LIVER FOCI AND c-myc/c-ras EXPRESSION, Paul Galand, Patricia Servais, Gérard Taton, Michèle Delronche, Annick Degeyter, Lab. Cytology & Exptl. Cancerology, School of Med. ULB, 1 rue Héger-Bordet, B-1000 Brussels, Belgium.
The present work addressed the question of the potential use of c-myc and/or c-ras expression level for characterizing high susceptibility (risk) towards carcinogenic action of a compound or of the carcinogenicity of the latter. We used therefore the rat liver model, checking female SD rats with a test dose of 5 mg DENA/100g b.w., 24h after one of the following pretreatments: 1-two-third hepatectomy (PH); 2-PH immediately followed by injection of 0.36mg indomethacin/100g b.w.; 3-100µg estradiol-17β (E2)/100g b.w. (which induced a slight, but significant increase in 3H-Thymidine labeling index); 4-Sham operation, solvent administration (controls). The incidence of foci of altered hepatocytes after 8-14 weeks in the controls and E2-pretreated animals was very low ; it was (as classically known) markedly increased by PH pretreatment (25 fold control level), an effect that was reduced 4-fold by indomethacin, which at the dose used did not modify the proliferative response to PH. There was no constant relationship between liver c-myc or c-ras expression (measured by Northern blot-cDNA hybridization) at the time of DENA application and the subsequent incidence of foci. DENA treatment by itself resulted in an early increase in liver content in c-myc, not in c-ras transcripts. This was not affected by indomethacin pretreatment. This suggests, that if an early increase in c-myc expression might be related to the induction of preneoplastic and neoplastic lesions, its role would not be sufficient, nor irreversibly determinant.

D 213 NEGATIVE REGULATION OF GROWTH FACTOR-RESPONSIVE TRANSCRIPTIONAL ENHANCER ELEMENTS, Michael J. Getz, Malayannan Subramaniam, and Lucy J. Schmidt, Department of Biochemistry and Molecular Biology, Mayo Clinic and Mayo Foundation, Rochester, MN 55905. Transcription of the c-fos proto-oncogene and the cytoskeletal actin genes is transiently induced within minutes of the addition of serum growth factors to a variety of cell types. Inhibitors of protein synthesis such as cycloheximide have been shown to dramatically potentiate the transcriptional response, an effect termed "superinduction." In this study we show that a synthetic copy of the c-fos serum-responsive enhancer element, or a related sequence associated with the human γ-actin gene, is sufficient to confer cycloheximide-dependent superinducibility upon a heterologous promoter. Moreover, treatment of quiescent cells with cycloheximide alone induces high levels of enhancer-dependent transcription in the absence of serum factors and at least some mutations which abolish cycloheximide inducibility also abolish serum inducibility. Since no other sequence elements appear to be required, these results imply that serum-inducible enhancer sequences are negatively regulated by one or more labile proteins. Such proteins may represent key intracellular determinants of growth and differentiation. (Supported by NIH grant CA33643 and by the Mayo Foundation.)

D 214 REGULATION OF EXPRESSION OF TGF-BETA TYPE 1 AND 2 IN NORMAL AND RAS INFECTED PRIMARY MOUSE KERATINOCYTES, Adam B. Glick*, David Danielpour*, Linda Dart*,David Morgan¥, Stuart H. Yuspa¥, and Michael B. Sporn*, * Laboratory of Chemoprevention and ¥ Laboratory of Carcinogenesis and Tumor Promotion, National Cancer Institute, Bethesda, Maryland 20892. The skin is an attractive model system to study the regulation of cell proliferation by positive and negative growth factors, and the disregulation which might occur during neoplasia. Transforming growth factor β types 1 and 2 are both potent growth inhibitors for many epidermal cell types including skin keratinocytes. We are investigating the role of these two members of the TGF-β family in normal skin physiology, and in the response to pharmacological agents which ar known to modulate epithelial growth and differentiation. The expression of TGF-β type 1 and 2 has been studied in primary epidermal keratinocytes isolated from newborn Balb/c mice, and in primary keratinocytes infected with a recombinant retrovirus containing an activated ras allele. The latter represent an in vitro model of initiated skin cells. TGF-β peptide and mRNA levels were determined for cells that were proliferating in low calcium medium, or induced to differentiate by elevated calcium, and for cells treated with retinoic acid and TPA. In both groups of cells we find a very close relationship between the effects of each agent on keratinocyte proliferation and the expression of TGF-β. For each treatment we also observed a significant difference between the normal and ras infected primary cells in terms of the specific TGF-β gene induced. This difference could have important implications for the disregulation of cell proliferation which occurs during skin carcinogenesis.

D 215 CHARACTERIZATION OF A SUPPRESSOR GENE CONTROLLED INHIBITOR OF ANGIOGENESIS, Deborah J. Good, Farzan Rastinejad and Noel P. Bouck, Department of Microbiology/Immunology and Cancer Center, Northwestern University, Chicago, Illinois 60611

Baby hamster kidney (BHK) cells transform from anchorage dependent and non-tumorigenic to anchorage independent and tumorigenic in a single step as a result of the inactivation of a cancer suppressor gene. One function of this suppressor gene is to control the elaboration of a 140kD polypeptide able to inhibit neovascularization in vivo. Active inhibitor was found in the conditioned media (CM) of cells only when they harbored an active suppressor gene. The inhibitor in CM eluted from a sizing column at the 120 to 140kD range, bound to a lentil lectin column and showed a 6.3% drop in apparent molecular weight when collected from tunicamycin-treated cells. Western analysis using rabbit antisera to purified inhibitor and polyclonal antibodies to known transformation sensitive glycoproteins with similar physical characteristics showed no cross-reactivity between inhibitor and fibronectin and only weak cross-reactivity between it and human collagen VI. However, amino acid composition of purified inhibitor showed it to be low in leucine and glycine, and lacking in hydroxylated proline and lysine characteristic of the 140kD collagen VI subunit. The amino acid composition was extremely similar to that of the 140kD fragment of thrombospondin. Monoclonal antibody A6.1 to human thrombospondin cross-reacted strongly with our inhibitor, and inhibitor antiserum recognized purified thrombospondin (kindly provided along with antisera by W. Frazier), indicating that a thrombospondin-like protein is responsible for the suppression-controlled inhibition of angiogenesis.

D 216 Correlation between expression of a TPA-induced gene (Phorbin) and Human colon cancer invasion. J.G. Guillem, M.F. Levy, L.L. Hsieh, M.D. Johnson and I.B. Weinstein, Dept. Surgery and Comprehensive Cancer Center, Columbia University, New York, N.Y. 10032

Our previous work on PKC and colon cancer has shown altered levels of PKC activity in human colon tumors as well as activation of PKC by colon tumor promoters such as bile acids. In order to further understand the role of PKC in colon carcinogenesis, we have analyzed the expression of phorbin, a gene induced by PKC activation, in a series of different stages of human colon tumors. Northern blot analyses of poly A^+ RNA from 10 colon tumor samples and their adjacent normal colonic mucosa indicated a uniform overexpression of phorbin RNA in all of the tumor samples when compared to their adjacent normal mucosa. Of particular interest was a correlation between abundance of phorbin RNA transcripts in the tumors and the extent of invasion (tumor/normal-fold increase= 2.0, 16.7 and 23.0 for Dukes A, B and C, respectively). Phorbin RNA was also abundant in a human colon cancer cell line (HT29). In parallel studies, we also examined the expression of other mitogen-responsive genes (c-myc, ODC and β-actin). All of the tumors displayed significant (mean 3.8-fold) increases in the level of c-myc RNA when compared to their adjacent normal colonic mucosa. About 47% and 16% of these tumor samples also showed increased levels of ODC (3.1-fold) and β-actin (1.6-fold) RNA, respectively. In contrast to phorbin expression, the increased levels of c-myc, ODC and β-actin RNA did not correlate with the extent of tumor invasion. Taken together, these results demonstrate a unique correlation between colon tumor invasion and phorbin RNA expression.

D 217 OVER-EXPRESSION OF p53 AND VL-30 IN MOUSE EPIDERMAL CARCINOMA CELLS, Kyung-An Han, Paul Rothberg and Molly Kulesz-Martin, Grace Cancer Drug Center, Roswell Park Memorial Institute, Buffalo, New York 14263.

Altered expression of known proto-oncogenes is being studied in an effort to understand the genetic basis of the stages of carcinogenesis. Tumor cell derivatives from three independent initiated cell lineages have been derived after 7,12-dimethylbenz[a]anthracene treatment of a common cloned parental cell line and characterized according to tumorigenicity. Northern blot analysis was done using a well-differentiated benign papilloma (O9RAT), a moderately differentiated squamous cell carcinoma (O5RATc) and an anaplastic carcinoma (O3RATc). The level of over-expression of certain genes in these tumor cell derivatives was determined by comparison with the normal parental cell line cultured under conditions favoring proliferation (in medium containing 0.02 mM Ca^{2+}) or differentiation (in medium containing 1.4 mM Ca^{2+}) to avoid differences due to the proliferative state of cells. The p53 and VL30 genes were found to be over-expressed 30-fold in O5RATc and O3RATc but not in O9RAT. The c-myc oncogene was over-expressed 10-fold in O5RATc only. There was no evidence of over-expression or 61st codon mutation of c-ras^H gene. Neither N-myc nor N-ras were detected in the tumor or normal cell lines. The well-differentiated squamous cell carcinoma derivative of the O9RAT papilloma lineage, and the initiated non-tumorigenic precursors of the O3RATc and O5RATc lineages are being examined in an effort to understand the relationship of altered expression of these oncogenes to biological stages of epidermal carcinogenesis. Supported by grants CA31101 and CA24538.

D 218 EFFECTS OF ANTISENSE RNA TO THE C-MYC GENE ON THE GROWTH OF 3T3 FIBROBLASTS IN VITRO
C.V. Herst and Steven Rosen, Dept.s of Tumor Cell Biology and Medicine, Section of Medical Oncology, Northwestern University, Chicago, Il. 60611.

The c-myc gene product has been demonstrated to be important in regulating cellular growth. Levels of c-myc mRNA rise rapidly after addition of serum to quiescent fibroblast cells. Both micro-injection studies with c-myc protein and transfection with c-myc-producing constructs have demonstrated a direct relationship between an increase in c-myc protein levels and increased rate of growth. Recently, the use of antisense RNA to c-myc has been used effectively to reduce the growth rate of both HL-60 and F-MEL cell lines in vitro. We chose to study the effects of antisense RNA to the c-myc gene in the 3T3 fibroblast cell line, a cell line possessing a normal phenotype. We transfected in a retroviral construct to produce antisense RNA to c-myc, and as a control, we used an antisense construct to the selectable marker gene, E.coli gpt, which we had previously transfected into the cell to make it gpt+. A decrease in both the growth rate and response to serum stimulation was observed in the antisense myc clones, but not in the control antisense gpt clones. Double-stranded RNA (dsRNA) hybrids of sense and antisense were detected in both nuclear and cytoplasmic RNA fractions in both cell clones. Neither antisense construct had any effect on the transcription of the respective endogenous sense gene, or other genes examined. The effects of antisense RNA on the amount of c-myc protein translated, and the relative half-life of the antisense transcript will be discussed.

D 219 SUPPRESSION OF THE NEOPLASTIC PHENOTYPE BY REPLACEMENT OF THE HUMAN RETINOBLASTOMA GENE PRODUCT IN RETINOBLASTOMA AND OSTEOSARCOMA CELLS,
Huei-Jen Su Huang, Jin-Yuh Shew, Phang-Lang Chen, and Wen-Hwa Lee, Departments of Pathology M-012 and Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093.

Mutational inactivation of the retinoblastoma susceptibility (RB) gene has been proposed as a crucial step in the formation of retinoblastoma and other types of human cancer. We have tested this hypothesis by introducing a cloned RB gene into retinoblastoma (WERI-Rb27) or osteosarcoma (Saos-2) cells that had inactivated endogenous RB genes. This was accomplished with a recombinant retrovirus containing RB cDNA (Rb virus). A control virus (Lux virus) was identical except the luciferase gene was used instead of the RB gene. Expression of the exogenous RB gene led to profound effects on many aspects of the neoplastic phenotype: 1) Starting at two weeks post infection, a majority (90-95%) of Rb virus-infected Saos-2 cells became flattened and greatly enlarged in average diameter (3-10 fold) compared to Lux virus-infected cells. These cells grew much more slowly than control cells, or stopped growing completely. With WERI-Rb27, moderately enlarged cells appeared four weeks after Rb virus infection, and the growth of these cells was also slower than control cells. 2) Soft-agar colony formation by Rb virus-infected Saos-2 cells was markedly reduced compared to Lux virus-infected cells. 3) Paired samples of 2×10^7 Rb or Lux virus-infected WERI-Rb27 cells were injected into either flank of seven nude mice. Four weeks after injection, six of seven mice formed tumors on the side injected with Lux virus-infected cells whereas zero of seven formed tumors on the side injected with Rb virus-infected cells. This demonstration of cancer suppression by a single gene provides direct evidence for an essential role of the RB gene in tumorigenesis.

D 220 THE v-abl ONCOGENE CONFERS A GROWTH-STIMULATING RESPONSE IN NIH3T3 CELLS TO TNF-α AS WELL AS RESISTANCE TO CYTOLYSIS,
Mien-Chie Hung, Ting Chung Suen, Richard, U. Rodriguz, and Jim Klostergaard, The University of Texas Cancer Center M.D. Anderson Hospital, Department of Tumor Biology, Box 79, Houston, Texas 77030

Tumor necrosis factor (TNF-α) is a monokine produced by macrophages and monocytes, and has been shown to have cytolytic, cytostatic or growth- stimulatory activity on transformed cells. However, the mechanism of these growth modulating activities of TNF-α is unknown. By studying the response of different oncogene-transfected cell lines to TNF-α, we showed that the oncogene *v-abl* confers resistance to the cytostatic and cytolytic activities of TNF-α as opposed to the parental NIH3T3 cells. Most interestingly, *v-abl* transfection also resulted in a growth-enhancing response to TNF-α up to the highest dose of 6,400 Units/ml. These altered properties were not due to the transformation event itself, since *EJ-ras* oncogene transfected NIH3T3 cells were susceptible to TNF-α. Moreover, EMT-6, a mouse adenocarcinoma cell line, which responded similarly to NIH3T3 cells, did not show growth-enhancement at high TNF-α dosages. Though resistant to the direct cytotoxic activity of TNF-α, the v-*abl* transfected cell line was effectively killed by macrophages, as were the other cell lines. This suggests tumor-cell killing by macrophages must involve mechanisms in addition to TNF-α.

D 221 LOSS OF HETEROZYGOSITY ON CHROMOSOME 13 AND STRUCTURAL ANOMALIES OF THE RETINOBLASTOMA SUSCEPTIBLE GENE IN OSTEOSARCOMA, Kanji Ishizaki, Junya Toguchida, Masao S. Sasaki, Mituo Ikenaga, Masayuki Sugimoto, Yoshihiko Kotoura, Takao Yamamuro, Radiation Biology Center and Department of Orthopedic Surgery, Faculty of Medicine, Kyoto University, Kyoto 606, Japan

We have analyzed the loss of heterozygosity on chromosome 13 and on other chromosomes in 33 osteosarcoma samples by using polymorphic DNA markers. In 32 informative cases, 20 tumors(63%) showed the loss of heterozygosity on whole or partial chromosome 13. All the detected deletions of chromosome 13 involve the region of 13q14 where the Rb gene is supposed to locate. The loss of heterozygosity was observed not only on chromosome 13 but also on other chromosomes with lower frequencies except chromosome 17. On chromosome 17, the loss of heterozygosity was observed with a frequency of 81%. Structural changes of the Rb gene in osteosarcoma were analyzed by Southern hybridization using the cloned cDNA of the Rb gene as a probe. We have observed structural anomalies of the Rb gene in 14 cases. They were either homozygous for deletion(7 cases) or heterozygous for deletion or rearrangement(7 cases). In some cases, regions of deletions or break points of the Rb genes were determined. Our data strongly confirm the hypothesis that the Rb gene is involved in the development of osteosarcoma.

D 222 MANIPULATION OF p53 EXPRESSION IN FRIEND ERYTHROLEUKEMIA CELLS, Penelope Johnson & Sam Benchimol, Dept. Medical Biophysics, U.of Toronto, & Ontario Cancer Institute/ Princess Margaret Hosp., 500 Sherbourne St., Toronto, Canada, M4X 1K9.

Aberrant expression of the p53 oncogene is seen in independent clones of transformed erythroid precursors arising in vivo from Friend virus-infected mouse spleens. Almost half of these clones do not express p53 due to mutation and loss of normal alleles. The remaining clones synthesize truncated p53-related proteins or p53 which appears abnormal since it has significantly increased stability and is not recognized by a monoclonal antibody (PAb246) recognizing an epitope on normal p53. It thus appears that there is selection against normal p53 expression during development of Friend erythroleukemia which may be satisfied equally by generation of immunological variants, truncated p53 proteins, or complete extinction of p53 expression. Stable and immunologically abnormal p53 protein may result from dominant negative mutations in the p53 gene. This model predicts that expression of normal p53 is not compatible with growth of malignant Friend clones. In order to test this hypothesis, we have attempted to express mutant and wild-type p53 in p53-negative Friend cells. A p53 gene isolated from a p53-producing Friend line, CB7, was co-electroporated with sequences conferring G418-resistance, into a p53-negative cell line. Resulting colonies were metabolically labelled to assess expression of the exogenous gene. p53 was expressed from the exogenous CB7 gene in all isolated colonies. The growth properties of these cells are currently being investigated. Since p53 from CB7 cells may encode an abnormal protein, similar experiments using wild-type p53 are in progress.

D 223 **The Effect of Translation Initiation Factor Phosphorylation on Protein Synthesis and Growth Control.** **Randal J. Kaufman, Monique V. Davis, J.W.B. Hershey. Genetics Institute Cambridge, MA and University of California, Davis, CA**

Phosphorylation of the alpha subunit of the eukaryotic translation initiation factor (eIF-2α) by the double-stranded RNA activated inhibitor (KAI) kinase correlates with inhibition of translation initiation. The importance of eIF-2α phosphorylation in regulating translation was studied by expression of specific mutants of eIF-2α in COS-1 cells. Transfection of plasmid DNA can activate DAI kinase and result in poor translation of plasmid derived mRNAs. Translation of plasmid derived mRNAs is improved by the presence of DAI kinase inhibitors or by the presence of non-phosphorylatable mutants (serine to alanine) of eIF-2α. The improved translation mediated by expression of the non-phosphorylatable eIF-2α mutant is specific to plasmid derived mRNA and does not affect global mRNA translation. In contrast, expression of a mutant eIF-2α harboring a serine to aspartic acid change, created to mimic the phosphorylated serine, inhibits global mRNA translation. These results substantiate the hypothesis that DAI kinase activation reduces translation initiation through phosphorylation of eIF-2α. Expression of mutant eIF-2α in stably transfected human 293 cells, results in altered growth properties. The effect of this mutant on translational control in these cells will be presented.

D 224 ONCOGENE EXPRESSION IN RADIATION-INDUCED CANINE LUNG TUMORS, Gregory Kelly[1], Paul R. Kerkof[1,2], and Patrick J. Haley[1], Lovelace Inhalation Toxicology Research Institute, P.O. Box 5890, Albuquerque, NM 87185, and [2]Dept. of Biology, University of New Mexico, Albuquerque NM 87131.

We obtained 10 lung carcinomas at necropsy from Beagle dogs that inhaled particles of $^{239}PuO_2$ as young adults as well as 2 canine lung carcinomas of spontaneous origin. These tumors were examined for the aberrant expression of up to 22 known oncogenes. Oncogene expression was examined by probing a battery of known oncogenes on a slot blot with labeled cDNA transcripts from primary tumor tissues. Densitometric scans of the resulting autoradiograms were used to identify those oncogenes expressed at elevated levels in these carcinomas. In 11 of 12 tumors tested, sequences hybridizing to the c-myc oncogene were expressed at levels 1.5 fold higher than sequences hybridizing to beta-actin. This level of oncogene expression was also observed in 9 of 12 tumors for one of more members of the ras family of oncogenes. In addition, 7 of 12 tumors examined express sequences which hybridize with oncogene clones of v-ros or c-met. The ros and met clones both code for oncogenes whose normal homologues are transmembrane proteins related to the insulin receptor. These results suggest that an autocrine stimulative mechanism may play an important role in the proliferative potential of these tumors. (Research sponsored by the U.S. Department of Energy's Office of Health and Environmental Research under Contract No. DE-AC04-76EV01013)

D 225 INVESTIAGTION OF RAS-INDUCED PHENOTYPIC CHANGES IN HUMAN SKIN FIBROBLASTS. A.R. Kinsella and L. Fiszer-Maliszewska, Paterson Institute for Cancer Research, Christie Hospital and Holt Radium Institute, Manchester, M20 9BX, UK. Using the defective retroviral shuttle vector system pZIPneo we have infected human skin fibroblasts with activated N-ras, c-myc (Miyamoto et al, 1985) and v-myb (Rushlow et al, 1982) genes coupled to the selectable marker for neomycin resistance. To date the presence of the activated N-ras gene has been shown to confer a markedly altered morphology and in rare instances foci of dense piled up cells typical of the transformed phenotype are seen. In two out of the eight N-ras infected subclones studied in detail, the presence of the activated N-ras gene seemed to induce a premature crisis and growth arrest. None of the clones have been shown to exhibit anchorage independent growth or produce tumours in mice, but they do show altered serum dependence. None of the clones showed marked chromosomal abnormalities except for an increase in ploidy in approximately 12 percent of the two cloned N-ras infected populations undergoing crisis. None of the subclones acquired immortality. In parallel the same human skin fibroblasts have been infected with an activated c-Ha-ras gene using an MPSV vector (Stocking et al, 1986). The subclones from these infections also exhibited altered morphologies which interestingly were distinguishable from both the normal and N-ras infected cultures. Skin fibroblast cultures infected with both myc and Ha-ras are being studied together with the effects of the ras oncogene on skin fibroblasts from cancer-prone individuals and cells already initiated with carcinogens.

D 226 ALTERATIONS IN PROTO-ONCOGENES AND GROWTH FACTOR RECEPTORS IN NON-SMALL CELL LUNG CANCER, Hironobu Koga, Robert Figlin, E. Carmack Holmes, Dennis Slamon, Division of Hematology-Oncology, UCLA School of Medicine, Los Angeles, CA 90024, and the Lung Cancer Study Group. Carcinoma of the lung is the number one cause of cancer related deaths in the United States. The majority of these malignancies (~75%) are non-small cell lung carcinomas (NSCLC), while ~25% are of the small cell variety (SCLC). The majority of the molecular research done in lung cancer has been done on SCLC. In this study, we evaluate alterations in proto-oncogenes, growth factors, and growth factor receptors in NSCLC. We studied a total of 62 primary NSCLC tumors from patients undergoing therapeutic resections in collaboration with the Lung Cancer Study Group (LCSG). Forty-four were male and eighteen were female. The mean age was 64.8 years (range 41-82). Histologic types were as follows; 32 adenocarcinomas, 23 squamous carcinomas, 6 large cell undifferentiated carcinomas and 1 tumor with mixed adeno and squamous features.

Among the genes studied for alterations at the DNA and RNA levels in the group were the epidermal growth factor receptor (EGFR), HER-2/*neu*, C-*myc*, N-*myc*, c-*abl*, c-*jun*, *int*-2 and c-*kit*. The EGFR gene was found to be amplified in 11 of 62 cases (18%). Six of these tumors were squamous, 3 were adenocarcinomas, 1 adeno-squamous and 1 large cell carcinoma. *Int*-2 amplification was observed in 3 squamous tumors and c-*kit* amplification was found in 1 adenocarcinoma and 1 squamous tumor. No significant alterations could be found in the HER-2/*neu* (c-*erb* B2), c-*myc*, N-*myc* or c-*abl* genes. Correlation between gene alterations at the DNA level and expression at the RNA level will be presented as well as association (if any) of gene alteration with clinical behavior.

D 227 PHORBOL ESTER-INDUCED FOCUS FORMATION IN A C3H/10T1/2 CELL LINE THAT CONSTITUTIVELY OVERPRODUCES PROTEIN KINASE C. Krauss, R.S., Housey, G.M., Johnson, M.D. and Weinstein, I.B. Institute of Cancer Research and Comprehensive Cancer Center, Columbia University, New York, NY 10032.
We have utilized a retroviral vector (pMV7) containing a full length cDNA encoding rat protein kinase C β1 (PKC) to construct a series of C3H/10T1/2 murine fibroblast cell lines that stably overproduce 3-11 fold greater PKC enzyme activity than parental cells or control cells that carry an integrated pMV7 vector that lacks the cDNA insert. 10T1/2-PKC-4, a line with an 11-fold increase in PKC activity was selected for detailed study. When grown in medium containing 10% calf serum, this line is morphologically altered, grows to 4-fold higher saturation density, and has decreased adhesiveness when compared to control cells. These cells also show constitutive alterations in the steady state mRNA levels of a PKC-regulated gene, TPA-R1. However, 10T1/2-PKC-4 cells do not have a transformed morphology, are incapable of growth in 0.3% agar (even in the presence of TPA), and are non-tumorigenic in nude mice. When 10T1/2 PKC-4 cells are cultured in medium containing 2.5% calf serum and 100 ng/ml TPA, numerous large, dense foci appear 2-3 weeks after the cells reach confluence. TPA is required for focus formation, and control cells do not form such foci under the same conditions. We have picked a number of such foci and are currently investigating their properties. The 10T1/2-PKC-4 cell line should be valuable for more precisely defining molecular events relevant to tumor promotion and multistage carcinogenesis. Supported by NCI grants CA02656 (to I.B.W.) and CA08346 (to R.S.K.).

D 228 THE ROLE OF THE EGF RECEPTOR IN TUMOR GROWTH SUPPRESSION BY EGF AND PUVA IN A431 CARCINOMA CELLS. Jeffrey D. Laskin and Fred H. Mermelstein, Department of Environmental and Community Medicine, UMDNJ-Robert W. Johnson Medical School, Piscataway, NJ. 08854
The photoactivated psoralens (PUVA) have been used in the treatment of a number of human malignancies. We have examined the mechanism by which psoralens mediate their antiproliferatve effects using A431 carcinoma cells. The activity of PUVA was compared to that of EGF, a well recognized growth inhibitor of these cells. Each of these inhibitors act by binding to distinct high affinity receptor proteins. EGF stimulates tyrosine kinase activity of its receptor, while PUVA induces cellular serine kinase activity. Treatment of A431 cells with both inhibitors resulted in phosphorylation of the EGF receptor. We found that serine phosphorylation of the EGF receptor in cells in response to PUVA caused an inhibition of its tyrosine kinase activity. Inhibition occurred as early as 45 sec following PUVA treatment and was dose-dependent in the nM concentration range. These effects were observed using psoralen analogs that do not cross-link DNA, supporting the model that a serine kinase mediates the action of PUVA. Taken together, these data suggest that modulation of tumor growth via the EGF receptor may occur by different mechanisms. Supported by NIH grant ES 03647.

D 229 PHORBOL ESTERS AND DIACYLGLYCEROL AS GROWTH INHIBITORS Arthur H.Lockwood, Maryanne Pendergast, and Suzanne K. Murphy. Harvard Medical School-LIJMC Research Consortium, & Philadelphia College of Pharmacy and Science.
We find that the tumor-promoting phorbol esters and diacylglycerol-activators of protein kinase C-enhance, rather than antagonize, cyclic AMP mediated growth inhibition and morphologic reversion of many tumor cells, including CHO and several ras and sis transformed lines. Both PMA and DAG synergistically stimulate the cAMP-dependent phosphorylation of cellular proteins with MW of 45 kd and 50 kd, togther with dephosphorylation of a 28 kd protein. Enhanced phosphorylation occurs on novel peptides.These phosphorylations are among the largest observed in living cells in response to second messengers or growth factors. Concurrently, there is a large and synergistic inhibition of polyphosphoinositide turnover. These results suggest that PKA and PKC may modulate growth and morphology by phosphorylation of the same subset of cellular proteins at unique sites. We also suggest that phorbol esters and diacylglycerols, usually considered to be tumor promoters & growth enhancers, may function as growth inhibitory factors and antagonize the action of certain oncogenes. Hence, the interplay of second messenger systems on growth stimulation or inhibition must vary with the normal or transformed cell in which they are studied. (Lockwood et. al. JCB 33, 237, 1987).

D 230 TRANSFER OF NORMAL GROWTH CONTROL AND DIFFERENTIATED FUNCTIONS TO HEPATOMA CELLS BY DNA-MEDIATED TRANSFECTION, Joseph J. Lucas and Lisa Johnson, Division of Basic Research, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colo. 80206
A rare product of the fusion of certain transformed mouse fibroblast and rat hepatoma cells is an epithelial-like cell which possesses the property of contact inhibition of growth to a remarkable degree. Cultures of cell lines established from such hybrids could be maintained as nearly perfect monolayers for 7 to 8 wks simply by occasional replenishment of growth medium. Cells appearing nearly identical to these synkaryon lines were also isolated after transfection of hepatoma cells with high molecular weight DNA isolated from growth-controlled hybrid cells. The growth-controlled transfectant lines arose with a frequency consistent with the notion that the transferred traits were due to the action of a single gene. The hybrid and transfectant lines shared a set of eight traits which clearly distinguished them from the parental hepatoma line. Most notable among these characteristics was the ability of the cells to form quiescent monolayers which then elaborated a complex network of structures resembling hepatic bile canaliculi. Compared to the parental hepatoma line, the transfectant lines also showed a marked increase in the relative resistance of DNA in isolated nuclei to digestion by DNase. It is proposed that the transferred gene responsible for the new traits might aptly be called an antioncogene and a strategy for identifying the action of such genes is presented. It is based upon the key observation that the morphology of colonies of growth-controlled transfectant cells was easily distinguished -- at the macroscopic level -- from colonies of the highly transformed, parental hepatoma cells. Large numbers of colonies could therefore be easily and rapidly screened.

D 231 MALIGNANT TRANSFORMATION OF HUMAN FIBROBLASTS BY TRANSFECTION OF ONCOGENES, J. Justin McCormick, Peter J. Hurlin, Daniel M. Wilson, Dennis G. Fry, and Veronica M. Maher. Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824-1316. Human fibroblasts in vitro have not been successfully transformed to malignancy by carcinogens, even though data indicate that carcinogen exposure is the cause of most human tumors. To understand why human fibroblasts in culture are so refractory, we have transfected them with oncogenes active in transformed rodent fibroblasts or in tumorigenic fibroblasts derived from human fibrosarcomas. We found that activated H- or N-ras genes flanked by suitable enhancer and promoter sequences caused normal human fibroblasts to express many transformed phenotypes, but the cells did not acquire an infinite lifespan and were not tumorigenic. When we transfected H-, or N-ras oncogenes in the same constructions used above or the v-K-ras gene into an infinite lifespan, near-diploid non-tumorigenic cell strain developed in this laboratory and designated MSU-1.1, distinct foci were observed. Cells from these foci formed progressively growing, invasive fibroblastic tumors in athymic mice. Immunoprecipitation analysis showed that these focus-derived cell strains, as well as the cells derived from their tumors overexpressed the H- and N-ras gene p21 proteins. The viral K-ras p21 was not overexpressed. However, unlike the H- and N-ras used, which contained only single mutations, the K-ras studied contained changes in codons 12 and 60. The H-ras oncogene in the same construction used above caused malignant transformation of two other infinite lifespan cell lines, KMST-6 cells and SV40-immortalized GM637 cells, suggesting that the infinite lifespan phenotype is required.

D 232 CHARACTERIZATION OF A NEW MOUSE CELLULAR GENE. HOMOLOGOUS TO THE FBR-MURINE SARCOMA VIRUS FOX SEQUENCES. Merregaert J., Van Hasselt F. and Michiels L., Department of Biochemistry, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium.
The Finkel-Biskis-Reilly Murine Sarcoma Virus (FBR-MuSV) is an osteosarcoma inducing retrovirus which has transduced c-fos sequences together with unknown cellular sequences called fox. In order to identify the nature of fox sequences, a cDNA library was constructed and hybridised with a fox specific DNA probe derived from FBR-MuSV proviral DNA. Fox cDNA consists of 486 nucleotides with an open reading frame encoding a putative protein of 133 amino acids. Both fox nucleotide sequence and deduced protein sequence were used to search for homologous products in the EMBL (version 14 of January 1988) and the SWISS PROT (version 6 of January 1988) data banks, but no matching was found. When compared to FBR-MuSV we found that 94 percent of the fox sequences were transduced and had suffered 7 point mutations, two of them changing the amino acid composition. Moreover, the fox sequences are transduced in the 3'-5' orientation giving rise to a possible anti mRNA synthesised "in vivo" by FBR-MuSV. Recently, Jenuwein and Müller (1987, Cell 48, 647-657) demonstrated that the v-fox sequences, which replace the 3' end of the c-fos gene in FBR-MuSV, cannot readily be substituted for by random sequences without loss of the enhanced transforming activity of FBR-MuSV. The authors suggested that weak homology in sequence between 3' fos protein and fox terminal FBR-MuSV fusion product may be functional. Our results suggest that v-fox may play a role in bone tumourigenesis by interfering with the normal c-fox function :
1. FBR-MuSV produces "in vivo" an anti c-fox mRNA
2. FBR-MuSV may initiate at the 3' LTR a v-fox encoding a mutated fox protein.

D 233 LOCALIZATION OF GROWTH FACTOR-INDUCIBLE AND REPRESSIBLE ELEMENTS IN THE TRANSIN GENE PROMOTER. Donna B. Miller, Lawrence D. Kerr, and Lynn M. Matrisian. Dept. of Cell Biology, Vanderbilt Univ. Sch. of Med., Nashville TN 37232 Transin is a secreted metalloprotease which is transcriptionally induced in rat fibroblasts by epidermal growth factor (EGF) and a number of oncogenes, including v-src, and H- and K-ras. The overexpression of transin in malignant and metastatic tumors and its matrix-degrading activity implies that transin is involved in tumor progression. We have previous demonstrated that transin mRNA is regulated at the transcriptional level, both positively by EGF and negatively by TGFß1. In order to investigate the molecular mechanisms regulating transin transcription, we have examined three transin promoter/chloramphenicol acetyltransferase (CAT) constructs for growth factor-inducible and repressible elements. Transient transfection of the construct p750TRCAT, containing transin promoter sequences from position -1 to -751, into Rat-2 fibroblasts demonstrated a 3-fold increase over low constitutive levels following EGF stimulation, a 9-fold induction with cotransfection of an H-ras construct, and a 1.8-fold increase with a c-ras construct. TGFß1, cAMP, and serum repressed EGF and ras induction of transin-mediated CAT utilizing this construct. p206TRCAT (-1 to -206) demonstrated no positive or negative inducibility, but was constitutively expressed at high levels. p84TRCAT position -84 was not sufficient for transin-mediated CAT expression, although this sequence contained the TATA and CAAT sequences, and the TPA-responsive element previously shown to be necessary for growth factor-induced transin expression. We conclude that an inhibitory element is present between positions -751 and -206, and that both growth factor inducible and repressible elements are contained within the first 750 bases of the transin promoter.

D 234 IDENTIFICATION OF cDNAs FOR POTENTIAL TUMOR SUPPRESSOR GENES FROM SYRIAN HAMSTER EMBYRO CELLS. Jeffry C. Montgomery, Junichi Hosoi, Paul E. Cizdziel, S. Jill Stowers-Hoffman, Roger W. Wiseman and J. Carl Barrett. Laboratory of Molecular Carcinogenesis, NIEHS, Research Triangle Park, NC 27709.
Loss of a tumor suppressor gene function is a distinct step in the *in vitro* multistage process of neoplastic transformation of Syrian hamster embryo (SHE) cells. Clonal variants have been isolated from immortal cell lines which have either retained ($supB^+$) or lost ($supB^-$) the ability to suppress the tumorigenicity of a benzo(a)pyrene transformed line in cell hybrids. cDNA probes prepared from $polyA^+$ RNA of $supB^+$ and $supB^-$ cells were used to screen 40,000 clones from a $supB^+$ Lambda Zap cDNA library for differential expression. Thirty one cDNAs which demonstrated preferential expression in the suppressor competent cells were isolated. These clones are derived from at least 3 independent genes as determined by insert cross-hybridization, similarities between restriction fragments in hamster DNA by Southern blots, and mRNAs on Northern blots. DNA sequence analyses revealed that 2 of these genes encode proα1(II) collagen and α1(IX) collagen which are normally expressed in chondrocytes. In order to isolate cDNAs from less abundant mRNAs, we are currently constructing libraries in the pcDpolyB mammalian expression vector which contains an f1 phage intergenic region. This vector allows preparation of single stranded phagmids, thereby facilitating the enrichment of $supB^+$ specific cDNAs by subtractive hybridization procedures. In addition to differential screening, this subtracted $supB^+$ library will be assayed for functional tumor suppressor genes by transfection and selection for cDNAs which inhibit tumor cell growth in media containing 1% serum.

D 235 INACTIVATION OF THE p53 GENE IS ASSOCIATED WITH CELLULAR TRANSFORMATION IN FRIEND VIRUS-INDUCED MURINE ERYTHROLEUKEMIA, Donald G. Munroe and Samuel Benchimol, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada, M4X 1K9.
During the progression of Friend virus-induced murine erythroleukemia, mutations in the p53 gene occur frequently and result in the loss of normal p53 expression. The p53 gene mutations present in certain Friend cell lines that fail to express p53 or synthesize truncated p53 polypeptides were described previously. We now demonstrate that an immunologically aberrant p53 protein is produced by other Friend cell lines with p53 alleles containing point mutations. Thus, loss of normal p53 expression is found in nearly all Friend erythroleukemias, indicating that p53 may belong in the class of "cancer suppressors" or "anti-oncogenes". In contrast to these in vivo data, gene transfer experiments have shown that variant forms of the p53 gene can act as dominantly transforming oncogenes. A model of p53-induced cellular transformation is presented which reconciles the apparently conflicting roles of p53 in these two experimental systems. We propose that functional inactivation of p53 is closely associated with cellular transformation in both systems, and that the oncogenic potential of transfected p53 alleles is the result of dominant negative mutations which disable endogenous p53 function.

D 236 POSITIVE AND NEGATIVE REGULATION OF CELL GROWTH BY HYDROGEN PEROXIDE GENERATED BY GROWTH SIGNALS. Kiyoshi Nose, Toshio Kuroki and Motoko Shibanuma, Department of Cancer Cell Research, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo, Japan.
We have previously reported that active oxigens generated by xanthine/xanthine oxidase activate Na^+/H^+ antiporter, as well as transcription of c-fos and c-myc and DNA synthesis in quiescent Balb/c 3T3 cells (Oncogene 3, 17, 1988). In the present study, we found that competence factors like PDGF and TPA stimulated the production of H_2O_2 in quiescent Balb/c 3T3 cells. Furthermore, when cells were treated with 0.1 - 0.2 mM H_2O_2, the amount of mRNA's for c-fos, JE and KC were increased to the level nearly comparable to that stimulated with serum. H_2O_2 stimulated DNA synthesis synergistically in the presence of insulin, but H_2O_2 alone did not. H_2O_2, serum and TPA all increased the phosphorylation of a protein species with MW of 78 kDa, pI of 6.1. When catalase was added at various times after stimulation of quiescent cells with PDGF or TPA, DNA synthesis was inhibited during 0 - 4 hr, but was stimulated during 8 - 10 hr. These results indicate that growth signals generate H_2O_2 which in turn regulates cell growth, either positively or negatively, depending on the conditions of cells.

D 237 GENETIC SUPPRESSION OF MALIGNANCY IN c-Ha-*ras* TRANSFORMED NIH3T3 CELLS BY A BACTERIAL ADENYLATE CYCLASE.
Kevin R. O'Driscoll*, Michiel M. Van Lookeren Campagne, I. Bernard Weinstein*, and Richard H. Kessin. Department of Anatomy and Cell Biology, *Institute of Cancer Research, Columbia University, 630 West 168th Street, New York, NY 10032.
To determine the effect of increasing cAMP production by genetic manipulation in *ras*-transformed cells, a recombinant retrovirus encoding the calmodulin-dependent adenylate cyclase from *Bordetella pertussis* (cycS) (Glaser *et al.* 1988 Mol.Microbiol. 2:19) was constructed using the pDOP neo vector (Schwartzbauer *et al.* 1987 PNAS 84:754). The packaged recombinant retroviruses DOP-cycS and DOP (vector control) were used to infect the NEJ-1 cell line, a derivative of NIH3T3 transformed by the EJ c-Ha-*ras* oncogene, which expresses a high level of $p21^{ras}$. Neo^+ clones were selected by growth in G418, expanded, and analyzed. Several NEJ-DOPcycS clones displayed a very flat reverted morphology, expressed cycS mRNA, and had decreased levels of EJ-*ras* mRNA and $p21^{ras}$ protein. Similar changes were not seen in the NEJ-DOP vector control or in the NEJ-DOPcycS clones that remained transformed. The saturation density of two revertant NEJ-DOPcycS clones was only one-fifth that of the parental NEJ-1 cells or NEJ-DOP cells, and in contrast to these control cells, which formed tumors within 1 week, they failed to form tumors in nude mice (test period 8 weeks). The phenomenon of reverse transformation by cAMP, hitherto only possible by pharmacological means *in vitro*, is here achieved genetically and its functional significance to *in vivo* tumorigenesis is demonstrated. This is the first system in which tumor suppression has been obtained with a gene whose function is well defined.

D 238 TRANSFER OF A NORMAL HUMAN CHROMOSOME 11 SUPPRESSES TUMORIGENICITY OF SOME BUT NOT ALL TUMOR CELL LINES, Mitsuo Oshimura, Minoru Koi, Hinoyuki Morita, Motoyuki Morita, Hideto Yamada and J. Carl Barrett, Laboratory of Cell Biology, Kanagawa Cancer Center Research Institute, Asahi-ku, Yokohama, Japan and Laboratory of Molecular Carcinogenesis, NIEHS, Research Triangle Park, NC 27709.
Stanbridge et al. first demonstrated the complete suppression of tumorigenicity of a human cervical cancer (HeLa) and a Wilms tumor (G401) cell line by the introduction via microcell fusion of a single human chromosome t(X;11). In order to determine whether other tumor cell lines are suppressed by chromosome 11, we performed chromosome transfer experiments via microcell fusion into various tumor cell lines, including SiHa (cervical carcinoma), A204 (rhabdomyosarcoma), HHU95 (uterine endometrial carcinoma), YCR (renal cell carcinoma) and On-1 (chemically induced Wilms tumor of rat). We first isolated a mouse A9 cell containing a single human chromosome 11 with integrated pSV2-neo plasmid DNA. Following microcell fusion of the neo-marked chromosome 11 into the various tumors mentioned above, we isolated clones which were resistant to G418, and performed karyotypic analyses and chromosome *in situ* hybridization to ensure the transfer of the chromosome. SiHa and A204 hybrid clones at early passages were non-tumorigenic in nude mice, and HHU95 was weakly tumorigenic, whereas the parental cells of each cell line were highly tumorigenic. On the other hand, YCR and On-1 hybrid clones were still highly tumorigenic following the introduction of chromosome 11. Thus, the introduction of a normal chromosome 11 suppresses the tumorigenicity of some, but not all, tumors suggesting that the function of the putative suppressor gene(s) on chromosome 11 is effective only in specific tumors.

D 239 THE TRANSCRIPTIONAL ACTIVITY OF A v-ras RESPONSIVE ELEMENT IS LINKED TO ONCOGENIC TRANSFORMATION, Russell D. Owen and Michael C. Ostrowski, Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710. We are investigating the molecular mechanisms by which ras oncogene expression causes the malignant transformation of cells. We present evidence demonstrating that NVL-3, a murine retrovirus-like gene, encodes a cis-acting transcriptional element that is modulated by Ha-v-ras and Ki-v-ras oncogenes. These results are obtained by analyzing the transient expression of chimeric plasmids that include NVL-3 information in various cell lines containing exogenous ras genes. Plasmids that include a 243 bp NVL-3 DNA segment exhibit 20-fold higher transcriptional activity in ras-transformed cells than in cells lacking activated ras genes. In a revertant, non-transformed cell line that displays high v-ras expression, the ras-responsive element is inactive. Taken together, this data defines a signaling pathway that connects the membrane-bound ras p21 product with the nucleus. Further, this pathway is linked to the ability of ras oncogenes to transform cultured cells. Using gel retention and footprinting assays, we have identified a nuclear factor which interacts specifically with the ras-inducible enhancer and have mapped DNA sequences responsible for mediating this interaction.

D 240 ANTI-PROLIFERATIVE EFFECTS OF HUMAN INTERFERON-ß ON HUMAN SMALL CELL LUNG CARCINOMA CELL LINES, Kathryn A. Pape and Georgia Floyd-Smith, Department of Zoology, Arizona State University, Tempe, Arizona 85287.

The effects of huIFNß on the expression of the *c-myc* oncogene and on cell growth rates have been studied in Small Cell Lung Carcinoma (SCLC) cell lines and in a non-SCLC cell line. NCI-H82 is a SCLC-variant cell line with 15-35 fold *c-myc* mRNA amplification due to *c-myc* DNA amplification (20-76 fold). NCI-H146 is a SCLC cell line with 6 fold *c-myc* mRNA amplification but no *c-myc* DNA amplification. NCI-H128, a SCLC cell line, and NCI-H125, a non-SCLC cell line, have control levels of *c-myc* mRNA and DNA. The effect of huIFNß treatment on [^{3}H] Thymidine incorporation into the DNA of these cells was studied. While huIFNß had no significant effect on [^{3}H] Thymidine incorporation in NCI-H128 cells and NCI-H125 cells, a 50% decrease in [^{3}H] Thymidine incorporation was observed in NCI-H82 cells and an 80% decrease was observed in NCI-H146 cells. To see if the growth inhibitory effects of huIFNß on the two cell lines could be correlated with decreased amounts of *c-myc* mRNA, northern blot analysis was performed. Levels of steady state *c-myc* mRNA were not found to be affected by huIFNß treatment. Furthermore, western blot analysis has shown huIFNß treatment is not effecting the *c-myc* protein levels in these cells. Therefore, the mechanism by which huIFNß is slowing the growth rates of NCI-H82 and NCI-H146 does not appear to be due to down-regulation of the *c-myc* oncogene, present in amplified amounts, in these cells.

D 241 TUMOR PROGRESSION IN COLORECTAL CARCINOGENESIS: ANEUPLOIDY AND RAS GENE MUTATION, Christos Paraskeva[1], Christopher J Marshall[2], Susan Powell[1] and Christine J Farr[2]. [1]Department of Pathology, University of Bristol, The Medical School, University Walk, Bristol BS8 1TD, [2]Chester Beatty Laboratories, ICR, Fulham Road, London SW3 6JB.
We have reported culture techniques for the isolation of human colorectal epithelial adenoma cell lines (Int.J.Cancer, 34,49-56,1984). One familial polyposis coli adenoma cell line, PC/AA, was diploid at early passage and after continuous passage with 3T3 feeders became immortal and aneuploid. Every cell of late passage PC/AA had an isochromosome 1(q). In a parallel experiment a 3T3 feeder independent immortal variant of PC/AA, designated PC/AA/FI, was isolated. Each cell of PC/AA/FI again has an isochromosome 1(q) similar to the late passage PC/AA. However with PC/AA/FI it is the second chromosome 1 of the homologous pair which is involved in the formation of the isochromosome 1(q). Both the immortal PC/AA late passage and PC/AA/FI have independent abnormalities of chromosome 1 indicating that chromosome 1 may be involved in in vitro immortalization. Two new sporadic adenoma cell lines, S/AN and S/RG have been isolated. Every cell of S/AN has a deletion on the short arm of chromosome 1 and is monosomic for chromosome 18. S/RG is monosomic for chromosomes 14,17,18 and 22. PC/AA and S/AN both have mutations in codon 12 of the c-K-ras gene as does an FPC carcinoma cell line, PC/JW. These results indicate that there are common molecular events in sporadic and hereditary colorectal cancer and that ras gene mutations can precede malignancy. They suggest a model wherein the adenoma carcinoma sequence involves activation of a dominant oncogene (K-ras) in a subpopulation of cells predisposed to malignancy perhaps by the loss of genes which normally suppress tumorigenicity.

D 242 BIOCHEMICAL PROPERTIES OF THE v-mos PROTEIN OF A VARIANT MOLONEY MURINE SARCOMA VIRUS, Elizabeth A. Putnam and Edwin C. Murphy, Jr., Department of Tumor Biology, Section of Molecular Virology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030. A variant of Moloney murine sarcoma virus (MuSV-SD) that induces rapidly fatal osteosarcomas in newborn rats has been studied. Blot hybridization of genomic DNA from the MuSV-SD cell line reveals three integrated MuSV-SD viral DNAs of 5.5, 5.0, and 4.3 kb. The 5.5 kb MuSV-SD DNA has been cloned and partially sequenced. The complete MuSV-SD v-mos sequence and predicted amino acid sequence shows a 96.5% and 97.1% similarity to the $P37^{mos}$ proteins of MuSV-124 and MuSV-Ht-1, respectively. In addition, the ATP binding sites of MuSV-124, MuSV-Ht-1, and MuSV-SD $P37^{mos}$ proteins are identical. To characterize the MuSV-SD-mos protein further, immunoblot analysis and immune-complex kinase assays were performed using anti-mos antisera. The immunoblot analysis demonstrated that similar levels of the v-mos protein were present in cells chronically infected with either MuSV-SD or MuSV-124; however, the serine kinase activity of the MuSV-SD-mos protein was vastly reduced compared to MuSV-124 $P37^{mos}$, in both autophosphorylation and transphosphorylation assays. Because the serine kinase activity of the v-mos protein has been postulated to be essential for the transforming capability of this protein, the minimal levels of MuSV-SD $P37^{mos}$ kinase activity detected raises questions as to the mechanism of transformation in this system. The cells infected with the MuSV-SD virus are highly transformed in culture by all conventional parameters, and cause rapidly fatal tumors in rats; therefore, it is possible that an alternate mechanism of transformation by this virus exists, possibly by the disruption of another regulatory cascade within the infected cells.

D 243 STUDIES OF THE RETINOBLASTOMA SUSCEPTIBILITY GENE IN VARIOUS HUMAN MALIGNANCIES, Peter T. Reissmann, Rei Takahashi, William F. Benedict, and Dennis J. Slamon, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024, and Center for Biotechnology, Baylor University, The Woodlands, TX 77381

The retinoblastoma susceptibility gene (RB) is a tumor suppressor gene whose deletion is associated with the development of retinoblastoma. Based on the observation that children with the heritable form of retinoblastoma frequently develop sarcomas as a second malignacy, bony and soft tissue sarcomas have also been found by several laboratories, including ours, to have deletions of the RB gene. Recently, human breast cancer and small cell cancer of the lung have also been reported to have deletions of the RB gene. In an attempt to define the scope of involvement of the this gene in human neoplasia, we have undertaken a survey of RB gene alterations in various human malignacies. DNA and RNA were extracted from fresh tumors and analyzed by Southern and Northern analysis. We have studied more than fifty breast cancers, more than thirty non-small cell lung cancers, and more than fifteen colon and rectal cancers. Data from these studies, as well as ongoing studies of other neoplasms will be presented.

D 244 Ras ONCOGENES IN HUMAN LUNG TUMORS ASSOCIATED WITH EXPOSURE TO CIGARETTE SMOKE, Steven H. Reynolds[1], Colleen K. Hunnicutt[1], Katie C. Brown[1], Ted Beattie[2], Ron Pero[2] and Marshall W. Anderson[1], National Institute of Environmental Health Sciences[1], Research Triangle Park, NC 27709; PMI-Strang Clinic and Kriser Lung Cancer Center[2], 55 East 34th Street, New York, NY 10016, The standard NIH/3T3 plate transfection technique, which selects exclusively for morphological transformation, and the NIH/3T3 transfection-nude mouse tumorigenicity assay, which depends only on tumorigenic potential, were both used to assay for potential oncogenes in human lung tumors. Seven of eight adenocarcinomas (AC) and two of three squamous cell carcinomas (SCC) were found to contain activated oncogenes by one or both of the assays used. Southern blot analysis identified the transforming gene as K-ras in four of the AC and H-ras in one of the SCC. The activating mutation in each of the K-ras genes was a GC to TA transversion in the first base of the 12th codon. A raf oncogene was detected in one of the AC. Three of the transforming genes did not hybridize with H-, K-, or N-ras, met, or raf. However Southern blot analysis with DNA from secondary nude mouse tumors derived from several primary nude mouse tumors exhibited consistent banding patterns with the human Alu probe. The Alu banding patterns suggest that the same oncogene was present in one AC and one SCC and a distinctly different gene was present in another AC. Thus, activated oncogenes were detected in a high percentage (9/11) of human lung tumors associated with cigarette smoking.

D 245 ANALYSIS OF A TRANSFORMATION-DEFECTIVE MUTATION OF ACTIVATED H-ras, Michael H.Ricketts, Glenda Durrheim, Marthinus J. van der Merwe, Honor North and *Arthur D. Levinson, MRC Centre for Molecular and Cellular Biology, P.O. Box 63, Tygerberg 7505, RSA and *Genentech Inc., South San Francisco, CA94080.
Specific point mutations in the three mammalian ras proto-oncogenes are associated with many human tumors. To define amino acids within the activated H-ras p21 protein essential for its transformation potential, point mutations were introduced into a cDNA expression vector encoding p21 with a pre-existing activating mutation (Val-12). The transformation potential of the encoded p21s was assayed by transfection of Rat-1 cells. Mutation of position 28 from phenylalanine to aspartic acid abolished the transformation potential of p21 (Val-12). The cDNA was constructed into a prokaryotic expression vector (driven by a tryptophan promoter) and the mutated p21 was isolated for analysis of guanine-nucleotide binding and GTPase activity. Hybridisation of radiolabelled GTP to extracts of E. coli Western-blotted onto nitrocellulose indicates that the Val-12 Asp-28 mutated p21 does not bind GTP. The results suggest that the conserved phenylalanine at position 28 contributes to the GTP-binding pocket of H-ras.

D 246 EXPRESSION OF c-myb PROTO-ONCOGENE IS STRONGLY ASSOCIATED WITH ESTROGEN RECEPTORS IN BREAST CANCER. **Martine Guérin, Michel Barrois and Guy Riou**, Laboratoire de Pharmacologie Clinique et Moléculaire, Institut Gustave Roussy, 94800 Villejuif (France).
Recent data from our laboratory have shown that overexpression of c-erbB-2, c-myc and/or epidermal growth factor receptor genes was associated with breast cancer progression. We extended this study to the c-myb proto-oncogene. Previous reports have shown that c-myb was expressed in only hematopoietic tumors suggesting that this gene plays a role in the development of these malignancies. However, no extensive studies of the c-myb gene have as yet been performed in solid tumors. In this report we analysed the expression of the c-myb gene in 85 specimens of breast adenocarcinoma at different clinical stages, obtained from untreated patients. Total RNAs were analysed by Northern and slot blot hybridization using a human c-myb probe. A 3.5 kb transcript band was clearly detected in 57 (67%) tumors while no transcripts could be detected in the 28 (33%) other tumors. The presence of c-myb transcripts was correlated with the most differentiated tumors ($p>0.03$), the presence of estrogen ($p=10^{-4}$) and progesterone ($p=10^{-4}$) receptors and the presence of pS_2 gene transcripts ($p=10^{-4}$). Expression of this latter gene was recently shown to be an estrogen-dependent marker in breast cancer. In conclusion, our data indicate that the expression of c-myb gene is associated with cancers of better prognosis. The determination of c-myb expression will allow the characterization of new classes of estrogen-dependent tumors which may respond differently to hormone therapy. Moreover, data also raise a question of more fundamental interest on the biological role of c-myb proto-oncogene in relation to hormonal status in breast cancer.

D 247 PROTOONCOGENE CHANGES IN MAMMALIAN CELLS TRANSFORMED BY RADIATION, R. Roots, T.C. Yang, University of California, Berkeley, CA 94720; and G. Grossi, University of Naples, Italy, and Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland. Midterm Syrian hamster embryo cell (secondary passage) were used in our studies of neoplastic cells transformed by radiation. We are using three criteria to evaluate possible changes in selected protooncogenes: (1) Restriction fragment length abnormalities, (2) Alterations in gene expression, and (3) Detection of genetic translocations or other abnormalities. Transformants were selected after clonal proliferation in soft agar. Subsequently neoplasia tests in nude mice were done. Transformants were followed by subculturing for prolonged periods post the initial isolation in soft agar. We have detected Pst-1 RFL differences and mRNA differences (both qualitatively and quantitatively) in the c-myc gene locus only well into the progression process after the initial isolation. Typically, certain chromosomal abnormalities -- a change from the normal 2N = 44 karyotype to a karyotype of 46-47 chromosomes -- occurs at some point into the progression process. In situ hybridization of protooncogene probes to the chromosome spreads is in progress. We intend to collect data from a number of independently isolated transformed cells in order to assess if a general (or universal) phenomenon exists. Work on other selected protooncogenes is begun. Our data do not agree with those reported by another group where trisomi in the number 7 chromosome of the Syrian hamster embryo cells was associated with radiation-induced cell transformation.

D 248 N-myc SUPPRESSES MHC CLASS I ANTIGEN EXPRESSION BY DECREASING BINDING OF AN ENHANCER-ACTIVATING PROTEIN, Anil K. Rustgi, Michael Lenardo, Andrea R. Schievella and René Bernards, The Cancer Center of Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, and The Whitehead Institute for Biomedical Research, Cambridge, MA 02142.

Amplification of the N-myc oncogene is associated with increased metastatic ability of neuroblastomas. Overexpression of the N-myc gene in neuroblastoma cells leads to a marked reduction in expression of MHC class I antigens. We have shown that the N-myc gene product supresses the expression of these antigens at the level of transcription: one element located within the first 138 basepairs upstream from the site of transcription initiation, the other between 138 and 190 basepairs upstream from the transcription start site. The latter sequence contains an enhancer that binds a nuclear factor, H2TF1, that stimulates the activity of the class I gene promoter by 6- to 8-fold. Based on a gel electrophoresis DNA-binding assay, we conclude the N-myc gene product diminishes the binding of this nuclear factor to its cognate binding site, thereby greatly reducing the activity of this enhancer. Experiments aimed at isolating the gene encoding H2TF1 will be presented.

D 249 CHROMATIN STRUCTURE OF AMPLIFIED AND SINGLE COPY C-MYC IN MALIGNANT FIBROUS HISTIOCYTOMA CELL LINES Jane C. Strandberg-Gibson, and Byron P. Croker, Department of Pathology, University of Florida, Gainesville, FL 32610. We previously reported that the c-myc gene was amplifed 2 to 11 times in 26 percent of malignant fibrous histiocytomas (MFH) examined (manuscript submitted). Increases in transcript levels of myc were observed to accompany gene amplification in these tumors. In order to identify potential areas of regulatory importance, we have examined the chromatin structure of the c-myc gene in human MFH cell lines with amplified (P3C) and single (UR-HCL-1) copies using DNAse I. Five DNAse I hypersensitive sites at identical locations were observed for UR-HCL-1 and a normal human fibroblast line HFF. One site located 5' to the first exon and 3' of promoter P0, and a second site located in the P2 promoter region are in accordance with DNAse I hypersensitive sites reported for myc in a Burkitt's lymphoma cell line BL 31 (Siebenlist, U., Henninghausen, L., Battey, J., and Leder, P. 1984. Cell 37: 381-391). Two other sites were located between exons 1 and 2 and the fifth site was 5' of P0. The amplified myc gene in the P3C cell line also had four of these sites, however the site 3' of P0 disappeared, and a new site in the 5' region of the first intron was seen. This site is located in an area with protein binding capabilities which seem to be lost as a result of point mutations in most Burkitt's lymphomas (Zajakc-Kaye, M., Gelmann, E.P., and Levens, D. 1988. Science 240: 1776-1780). These data suggest that gene dosage effects alone are not responsible for increases in myc transcript observed in MFHs. Also, amplified myc in MFHs my be regulated differently than a single copy myc through the loss of one regulatory site near the P0 region, and the gain of another site in the 5' region of the first exon.

D 250 **Oncoproteins as components of signal transducing networks**

Axel Schönthal*, J.Feramisco*, H.Ponta, H.J.Rahmsdorf, P.Herrlich. Institute for Genetics, Nuclear Research Center, 7500 Karlsruhe, W.-Germany; *UCSD Cancer Center, San Diego, CA.

(Proto)oncogene products are assumed to act as components of a cell growth controlling network which transfers signals arriving at the cell surface to the nucleus. Elucidating their hierarchical order and their interactions will lead to the understanding of normal growth control as well as carcinogenesis.

We show that the activation of oncoproteins localized within different compartments of the cell leads to changes in gene expression: e.g. the fos and jun oncogenes as well as the collagenase gene are activated. Sufficient and necessary for the activation of the collagenase gene is the same promoter element which is needed for TPA induction of this gene. The nuclear fos and jun oncoproteins themselves are essential components of this network: inhibition of their expression by anti-sense RNA abolishes activation of the collagenase gene by oncogenes, TPA or growth factors. Furthermore these two oncoproteins act as transrepressors: expression of both leads to a repression of the c-fos promoter.

D 251 REVERSIBLE ANTIPROLIFERATIVE/ANTIONCOGENIC EFFECTS OF A PLANT LECTIN. Gail Seigel and Raphael Mannino, Albany Medical College, Albany, NY 12208 Regulatory processes controlling cell growth and gene expression are closely intertwined. When highly proliferative SV40–transformed 3T3 cells are treated with the lectin derivative succinylated concanavalin A (SCA), reversible down–regulation of cell growth and gene expression occurs. Changes in SV40 large T antigen and P53 oncogene protein accompany SCA–induced simulation of density–dependent growth inhibition. Specifically, SV40 large T antigen and P53 proteins are greatly reduced throughout the cell cycle, as detected in fixed cells by flow cytometry and modified ELISA techniques. The magnitude of SCA effects can be increased by higher SCA concentrations, or by lengthening duration of SCA treatment. In addition, SCA effects on SV40 large T antigen can be reversed after 1 hour incubation of SCA–inhibited cells with alpha methyl–mannoside. Thus, the return to high levels of detectable SV40 large T antigen appears to be an early event in SV3T3 cells resuming the growth pattern of a transformed phenotype.

Currently, Northern analysis of SV40 and P53 mRNA's is underway to characterize the status of these messages in relation to the protein products expressed during SCA treatment. These studies will help determine whether control of these proteins' expression resides at transcription or translation.

D 252 ISOLATION AND CHARACTERIZATION OF MITOTIC INHIBITOR FROM HUMAN AMNION CELLS IN TISSUE CULTURE, Chandrashekhar N. Shenoy, Shirin M. Marfatia and Kamalakar A. Chaubal, Biophysics Unit, Cancer Research Institute, Parel, Bombay 400012, INDIA.

The growth of cells is a well regulated phenomenon depending on several factors which play important roles in controlling the overall growth of normal cells. Though the precise mechanism of growth regulation is not known, several substances affecting growth (promotion and inhibition) have been isolated, few theories have been postulated and evidence is accumulating for the definite role played by growth factors and inhibitors.

In our observations when the used up medium of Human Amnion cells in tissue culture was fractionated, a fraction having absorption at 260 mμ occurred consistently. The addition of this fraction to Human Amnion cells caused inhibition of mitosis without toxicity. Further experimentation showed this fraction to be specific in action towards its own cell type and this action was reversible. The effect of the fraction on macromolecular synthesis is being studied using autoradiography and cytophotometry. Investigations using microplanimetry showed that the mitotic inhibitory fraction caused enlargement of cells. The fraction consists of peptide and deoxyribonucleotides. The molecular weight of the fraction was 1500 Da. The fraction was found to be heat stable. The mitotic inhibitory activity of the fraction was destroyed by Trypsin. From the staining procedures it was inferred that the peptide moiety has N-terminal block.

D 253 $p185^{HER2}$ MONOCLONAL ANTIBODY HAS ANTIPROLIFERATIVE EFFECTS IN VITRO AND SENSITIZES HUMAN BREAST TUMOR CELLS TO TUMOR NECROSIS FACTOR, H. Michael Shepard, Gail D. Lewis, Marcy Winget, Robert M. Hudziak and Axel Ullrich, Departments of Pharmacological Sciences and Developmental Biology, Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080. The HER2 gene encodes the EGF receptor-like human homolog of the rat *neu* oncogene. Amplification of this gene in primary breast carcinomas has been shown to correlate with poor clinical prognosis in node-positive cancer patients. We show here that a monoclonal antibody directed against the extracellular domain of $p185^{HER2}$ specifically inhibits the growth of breast tumor derived cell lines overexpressing the HER2 gene product and prevents HER2 transformed NIH 3T3 cells from forming colonies in soft agar. Furthermore, resistance to the cytotoxic effect of TNF-alpha, which has been shown to be a consequence of HER2 overexpression, is significantly reduced in the presence of this antibody.

D 254 MALIGNANT TUMOR INDUCTION BY V-SRC : SUFFICIENCY OF ONCOGENE WHEN EXPRESSED FROM UNIQUE VIRUS VARIANT. A. W. Stoker, M. Sieweke, and M. J. Bissell. Laboratory of Cell and Molecular Biology, Lawrence Berkeley Laboratory, Berkeley, CA.
Fibrosarcoma initiation and progression are characteristics of the acutely oncogenic Rous sarcoma virus ; the v-*src* oncogene is a sufficient genetic determinant for malignant growth under these conditions. When expressed alone, in a non-viral context, v-*src* has previously been shown to induce fibrosarcomas which regress and are not fully malignant in avian tissue. We have addressed whether v-*src*, when expressed from a replication-defective retrovirus, is capable of inducing a fully neoplastic phenotype. Using an avian vector packaging cell line and a v-*src* retroviral vector, replication-incompetent transforming viruses were obtained and innoculated into day 2-4 chick wing webs. Rapidly growing fibrosarcomas arose within 7-14 days; tumors did not regress but grew to kill the host in 2-4 weeks. Secondary tumors were found in livers and lungs in approximately 10% of birds. The virus innoculum could be reduced to less than ten infectious particles, and tumors were still obtained. Examination of tumor DNA's confirmed their clonal or oligoclonal nature, and explants from tumors were predominantly free of replicating virus. These data demonstrate that the v-*src* oncogene alone is sufficient for the induction of malignant fibrosarcomas. An unexpected finding was that a truncated virus variant had been selected for in most tumors. The structure of the virus suggested that high v-*src* expression levels may be required before tumor induction occurs. We are currently examining the source of this variant virus, and the pattern of its expression in tumors.

D 255 THE IDENTIFICATION OF TWO NUCLEAR FACTORS BINDING TO A NEGATIVE ELEMENT OF HUMAN C-MYC GENE : THE FOS/JUN AND THE OCTAMER BINDING PROTEIN SHARE BINDING SITES. Masato Takimoto, John Quinn, A. Rosella Farina, Louis Staudt and David Levens. Laboratory of Pathology and Metabolism Branch, National Cancer Inst., Bethesda, MD 20892.
There is a negative transcriptional element approximately 300bp upstream of the human c-myc promoter P1. Within this element, a 26bp-region was protected from digestion using a DNase I footprint assay. Two specific DNA-protein complexes were identified by gel retardation assay, using HeLa cell nuclear extract and an oligonucleotide which spans the footprinted region. The results of exonuclease and chemical footprint analyses suggest that the binding sites for these two specific complexes are almost entirely overlapping on the c-myc DNA sequence. One of the complexes is eliminated by oligonucleotide competitors which have known AP-1 binding sites. This complex is specifically inhibited by anti-fos antibodies, showing that it contains the fos/jun(AP-1) complex. c-myc DNA immobilized on beads specifically precipitated the material immunoreactive with anti-fos antibody. The other complex is eliminated by an oligonucleotide competitor bearing an octamer motif sequence more efficiently than by c-myc oligonucleotide. Purified ubiquitous octamer binding factor formed complex with the c-myc oligonucleotide comigrating with the complex formed by crude nuclear extract. The demonstration that two different factors, each distinct consensus recognition sequences, can bind to the same element suggests that multiple modes of regulation can be achieved by altering the abundance of the factors. Because both fos/jun and octamer binding protein can bind to positive and negative element we infer that certain mechanistic feature may be shared between transcriptional activation and repression.

D 256 CHROMOSOMAL LOCALIZATION OF THE REGIONS LOSING HETEROZYGOSITY ON CHROMOSOME 17 IN OSTEOSARCOMA , Junya Toguchida[1,2], Kanji Ishizaki[2], Yusuke Nakamura[3], Masao S. Sasaki[1], Mituo Ikenaga[1], Mitsuo Kato[1], Masayuki Sugimoto[2], Yoshihiko Kotoura[2], Takao Yamamuro[2], [1]Radiation Biology Center and [2]Department of Orthopaedic Surgery, Faculty of Medicine, Kyoto University, Kyoto 606, Japan, [3]Howard Hughes Medical Institute, University of Utah Health Science Center, Salt Lake City, Utah 84132
We recently reported the loss of heterozygosity on chromosome 13 and structural anomalies of retinoblastoma susceptibility (RB) gene in osteosarcoma. In addition to chromosome 13, some other chromosomes were found to be lost their constitutional heterozygosities in many osteosarcoma tumors. Among those chromosomes, the heterozygosity on chromosome 17 was lost with a highest frequency. We further investigated the frequency of these changes and also tried to localize the involved regions using 10 polymorphic markers on chromosome 17. We found that 26 of 32 osteosarcomas analyzed (81%) lost the heterozygosity on chromosome 17 and that the involved regions differed in each cases, being from a whole chromosome 17 to distal part of the short arm. The common region was 17p13. These results suggest that another recessive oncogene located in this region may be involved in the development of osteosarcoma.

D 257 DNA-PROTEIN INTERACTIONS ASSOCIATED WITH THE cKI-RAS PROMOTER REGION, Eric K. Hoffman, Stephen P. Trusko and Donna L. George, Howard Hughes Medical Institute, University of Pennsylvania, Philadelphia, PA 19104.

The potential transforming activity of the cKi-ras oncogene can be activated by genetic changes resulting in an altered protein product or overexpression of an otherwise normal product. To gain insight into how transcription of this gene is regulated, we have characterized the cis-acting elements associated with the 5' flanking region of the mouse cKi-ras gene. Transient expression studies utilizing the bacterial chloramphenicol acetyltransferase (CAT) gene were used to initially localize regions important for cKi-ras promoter function. Stepwise deletion of 5' sequences resulted in a gradual decrease in promoter activity suggesting that the promoter is comprised of multiple cis-active elements. The most critical region for promoter activity was found to lie within a 160 bp restriction fragment which contains two potential Sp1 binding sites and a CT-rich stretch of nucleotides. Using gel mobility shift and DNAase I protection assays, we identified DNA domains within the 160 bp fragment which bind nuclear proteins and most likely represent transcriptional regulatory elements. Some of the sequence elements within this fragment are similiar to those contained within the promoter regions of other housekeeping or growth control genes and may act as binding sites for regulatory factors common to a set of such growth-control genes.

D 258 **MOLECULAR ANALYSIS OF THE RAT ORNITHINE DECARBOXYLASE GENE.**

H.J. van Kranen,H. van Steeg, C.T.M. van Oostrom, J.W.M. Martens, H.M. Hodemaekers,, C.F. van Kreijl. National Institute of Public Health and Environmental Protection, Laboratory of Carcinogenesis and Mutagenesis, 3720 BA Bilthoven, The Netherlands.

Ornithine decarboxylase (ODC) is the first and rate limiting enzyme in the pathway of polyamine biosynthesis and its gene is subjected to control by a wide variety of stimuli for cell proliferation. In order to study expression of the ODC-gene in relation to tumor promotion and cell growth, we cloned the functional ODC gene (clone pODC821, 7776 bp BamHI fragment) from total rat liver DNA. Comparison of the ODC cDNA sequence and the genomic sequence reveals that the gene consists of 12 exons and 11 introns. Two introns were found in the region encoding the (303/304 nucleotide) 5' untranslated leader sequence. The first of these is also the largest of all introns, comprising 1877 bp. The organization of the protein coding part of the ODC-gene is strongly conserved between mouse and rat.Intron lenghts, however vary between rat and mouse. At the 3' end of the gene two alternate polyadenylation sites were found.The capsite of the rat ODC gene is located 1157/1158 bp downstream of the 5' BamHl site of pODC821. In a region 360 bp upstream of the cap-site several putative promotor- and enhancer elements are discernable e.g. TATA-box, GC-boxes, BLE, TRE, CRE. Interestingly a conserved AP-1 binding site is also found in intron-1. To examine the ODC promotor region, we fused parts of the ODC-gene to the coding region of the bacterial CAT gene and tested these constructs in Rat-1 cells. The shortest construct displaying full constitutive expression of CAT contains 105 bp upstream of the capsite. Individual control elements are now being delineated by analysis of promotor mutations.

D 259 LONG-RANGE PHYSICAL MAPPING OF THE WILMS' TUMOR AND ANIRIDIA LOCI, Michael M. Weil, Duane A. Compton, Louise C. Strong, and Grady F. Saunders, Department of Biochemistry and Molecular Biology and Department of Pediatrics, The University of Texas M. D. Anderson Cancer Cente,r Houston, TX 77030.

Alterations in chromosomal band 11p13 are associated with Wilms' Tumor (WT) and one form of aniridia (AN2). Cloning the sequences involved in Wilms' tumor and AN2 would lead to an increased understanding of the molecular events important in their etiology. We have constructed a long-range (13 Mb) physical mapof 11p13 with the goal of precisely defining the area within 11p13that can contain the sequences of interest. This map has been used to determine the extent of visable submicroscopic deletions affecting constitutional DNA in Wilms' tumor and aniridia patients. The results have allowed us to focus our search to AN2 and the WT supressor gene to specific DNA fragments defined by rare cutting restriction enzymes.

D 260 COMBINED EXPOSURES TO POLYCYCLIC AROMATIC HYDROCARBONS MODIFY SURVIVAL AND NEOPLASTIC TRANSFORMATION. R.L. Wells, C.C. Chen, and M.M. Elkind, Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, CO 80523.

PAH's are ubiquitous environmental contaminants which induce pleiotropic biological responses. We are examining the combined effects of these carcinogens on the colony forming ability and transformation frequency of mouse embryo C3H 10T½ cells. Nontoxic exposures of 3-methylcholanthrene (3MC), benzo(e)pyrene [B(e)P], and α-naphthoflavone (αNF) are all antagonistic to the lethal effects induced by either 7,12-dimethylbenz(a)anthracene (DMBA) or benzo(a)pyrene [B(a)P]. The degree of antagonism increases with increasing concentration with αNF being the most effective on a molar basis. Under certain conditions, we have observed a rescue (reversal) from lethality. For instance, when cells are exposed to DMBA for 24 hours and then αNF is added to the medium for an additional 24 hours, survival increases to levels above those for DMBA alone. The reversal is quantitatively dependent upon serum concentration as well as serum lot and source (fetal bovine vs. calf serum). Preliminary results also indicate an antagonistic action by these same modifier PAH's on DMBA induced transformation. These observations are being extended to include measurements of adduct persistance and aryl hydrocarbon hydroxylase induction. (Work supported by the National Cancer Institute, Grant No. CA 47497.)

D 261 DIFFERENTIAL LOSS OF HETEROZYGOSITY IN HUMAN NON-SMALL CELL BRONCHOGNEIC CARCINOMA, Ainsley Weston, James C. Willey, Rama Modali, Haruhiko Sugimura, Bret Light, Aage Haugen, James Resau, Elizabeth McDowell, Benjamin F. Trump, Dean L. Mann and Curtis C. Harris, NCI, National Institutes of Health, Bethesda, MD 20892 and Department of Pathology, University of Maryland, Baltimore, MD 21201
Human lung cancer accounts for more than 125,000 deaths annually in the U.S.A., most are smoking related. Since tobacco smoke contains a complex mixture of clastogenic agents it is likely that a broad spectrum of allelic deletion will be a feature of this family of diseases. In order to examine this question we have examined 54 non-small cell bronchogenic carcinomas with 13 polymorphic molecular markers. Our results show examples of loss of heterozygosity in at least one case for each of the loci distributed on six chromosomes, that were studied. Among 23 squamous cell carcinomas loss of heterozygosity was generally more frequent than among 23 adenocarcinomas or 8 large cell carcinomas. Shortest region of overlapping deletion analysis at six polymorphic loci that map to chromosome 11 revealed evidence for two distinctly different regions of loss of heterozygosity, (11p 13 and 11p 15.5). Loss of heterozygosity for chromosome 17p (D17S1) was also found to be consistent in squamous cell carcinomas (8/9) but not adenocarcinomas (2/11). These data suggest that a more general mechanism possibly involving the loss of tumor suppressor genes operates in this adult tumor type than has been previously described for some childhood malignancies, e.g. retinoblastoma, Wilms' tumor and rhabdomyosarcoma.

D 262 ISOLATION OF FUNCTIONAL HUMAN TUMOR SUPPRESSOR GENE cDNAs BY TRANSFECTION INTO SYRIAN HAMSTER TUMOR CELLS, Roger W. Wiseman, Esther W. Hou, Lois A. Annab and J. Carl Barrett. Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.
Several lines of evidence indicate that loss of a tumor suppressor gene function is a critical step in the neoplastic transformation of Syrian hamster embryo (SHE) cells *in vitro*. Studies of SHE cell hybrids formed between preneoplastic variants and tumor lines have demonstrated that at least three complementation groups exist for tumor suppression in this system. We have utilized tumor lines from each complementation group as gene transfer recipients with a cDNA library prepared from normal human fibroblast mRNAs in the pcD2 expression vector (generously provided by Dr. Hiroto Okayama, NIH). After G418 selection, transfectants which replicated in media containing BUdR and 1% serum were killed by near UV light treatment, while growth suppressed transfectants survived and were cloned after growth in normal media. Transfection of the cDNA library into benzo(a)pyrene- or v-H-*ras*/v-*myc*-transformed lines yielded only small increases in survival in the 1% serum/BUdR/near UV light selection relative to control cultures treated with the pcDneo vector alone. In contrast, transfer of the cDNA library into spontaneously transformed BHKA tumor cells increased survival by up to 10-fold. At least four independent cDNA-induced revertant BHKA clones have been isolated which demonstrated up to 1000-fold suppression of anchorage independence relative to BHKA cells. The suppressed phenotype of these primary revertants has been transferred in secondary transfections. Progress in the molecular cloning and characterization of the biologically active cDNAs will be reported.

D 263 **ACTIVATION OF THE K-RAS PROTOONCOGENE IN SPONTANEOUSLY OCCURRING AND CHEMICALLY INDUCED LUNG TUMORS OF THE STRAIN A MOUSE.** Ming You, Urs Candrian, Robert R. Maronpot, Gary D. Stoner, and Marshall W. Anderson NIEHS, Research Triangle Park, NC 27709; MCO, Toledo, OH 43699; The strain A mouse has a high incidence of spontaneous lung tumors,and is susceptible to lung tumor induction by chemical carcinogens. By utilizing transfection assay and Southern blot analysis, we have detected an activated K-ras gene in the DNAs of both spontaneously occurring and chemically induced lung tumors of strain A mice.The point mutations in the spontaneous lung tumors were in both codon 12 (60%) and codon 61 (30%). In contrast, 100% of the mutations in the K-ras gene detected in MNU-induced lung tumors and 93% of the mutations in the K-ras genes detected in BP-induced lung tumors were in the 12th codon,while 90% of the mutations in the K-ras genes detected in EC-induced lung tumors were in the 61st codon. The selectivity of mutations in the K-ras oncogene observed in chemically induced tumors, as compared to spontaneous tumors, suggests that these chemicals directly induce point mutations in the K-ras protooncogene. These data indicate that the strain A mouse lung tumor model is a very sensitive system to detect the ability of chemicals to activate in vivo the K-ras protooncogene in lung tissue.

Differentiation, Development and Carcinogenesis

D 300 PROTEIN KINASE C - MEDIATED HISTONE H2B PHOSPHORYLATION SPECIFIC TO MONOCYTIC DIFFERENTIATION OF PROMYELOCYTIC HL - 60 LEUKEMIA CELLS, C. Stuart Baxter and D. Gayle DeBord, Department of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH 45267.
In order to examine the role of nuclear histone phosphorylation in regulation of the pathway of promyelocytic HL-60 leukemia cell differentiation, cells were labelled with [^{32}P]phosphoric acid and histones fractionated by two-dimensional polyacrylamide gel electrophoresis. The monocytic inducer and protein lipase C activator 12-O-tetradecanoylphorbol-13-acetate (TPA) was found to specifically stimulate phosphorylation of histone H2B in a concentration-dependent manner. At a concentration of 100 mM, H2B phosphorylation was stimulated 2.3-fold after 4 h. A second monocytic inducer 1,25-dihydroxycholecalciferol (100 mM) also induced phosphorylation specifically in histone H2B. In contrast, the granulocytic inducers DMSO (1.5%) or retinoic acid (1μM) did not increase phosphorylation in any histone species. Stimulation of histone H2B phosphorylation was also induced by the diacylglycerol sn-1,2-dioctanoylglycerol (150um) and by phopholipase C (0.2units/ml). These findings therefore suggest that histone H2B phosphorylation specifically regulates monocytic differentiation of HL - 60 cells and that this process is further mediated by protein kinase C.

D 301 MULTISITE PHOSPHORYLATION OF THE *myb* AND *jun* /AP-1 NUCLEAR PROTEINS

W.J. Boyle[1], J.R. Woodgett[2], M. Karin[3] and T. Hunter[1], [1]The Salk Institute for Biological Studies, La Jolla, CA, [2]The Ludwig Institute for Cancer Reasearch, London, England and [3]The Department of Pharmacology, School of Medicine, UCSD, La Jolla, CA.

The Ultimate fate of mitogenic signal transduction, triggered by protein kinases activated in respone to signal reception, is to initiate a cascade of nuclear functions that culminate in cell division. The nuclear protein targets of signal transduction, once activated, may directly participate in crucial nuclear functions that regulate either early (G1) and late (G2) gene expression, replication and segregation of DNA, or disassembly and reassembly of the nuclear membrane and peripheral lamina. To learn more about nuclear signal transduction pathways, we are studying the phosphorylation of key nuclear proteins with a known role in cell growth control. Obvious choices for analysis are the nuclear products of the viral and cellular oncogenes, such as *fos*, *myb*, *myc*, and the newly added member *jun*/AP-1. This group of proteins is likely to be the final recipients of transduced signal, which in turn may act as direct effectors of mitogenesis. We are currently investigating the role of nuclear protein phosphorylation in cell growth control using two distinct but suprisingly related systems: the *myb* protein during hematopoiesis and leukemogenesis, and the *Fos* and *jun*/AP-1 nucleoprotein complex during TPA-induced transcriptional activation. Both the *myb* and *jun*/AP-1 proteins are phosphorylated *in vivo* on serine and threonine residues that are phosphorylated by the glycogen synthase kinase-3 (FA-kinase) *in vitro*. We will present evidence that the phosphorylation of *myb* and *jun*/AP-1 occurs via a process fundamentally similar to the regulatory multisite phophorylation of glycogen synthase.

D 302 CHARACTERIZATION OF HUMAN c-REL cDNAs. E. Brownell, N. Mittereder,* and N.R. Rice.* Molecular Therapeutics, Inc., West Haven, Ct. and * Laboratory of Molecular Virology and Carcinogenesis, Bionetics Research, Inc., Frederick, Md.

We have previously described a genomic DNA fragment that contains two exons of the human c-rel protooncogene. Using this fragment as a hybridization probe, we screened a cDNA library made from the Daudi Burkitt lymphoma cell line, which expresses high levels of c-rel transcripts. Two cDNA isolates were characterized, one of which apparently contains a complete coding sequence. This cDNA also contains a new exon that is missing in the second cDNA isolate. The unusual structure of this new exon, and its potential functional role will be discussed.

Research sponsored by the National Cancer Institute, DHHS under contract No. NO1-CO-74101 with Bionetics Research, Inc.

D 303 EFFECTS OF PHORBOL ESTERS AND INSULIN ON ORNITHINE DECARBOXYLASE TRANSCRIPTION AND PHOSPHORYLATION OF NUCLEAR PROTEINS IN RAT HEPATOMA CELLS, Andrew P. Butler, Penny K. Mar and William B. Cohn, Science Park—Research Division, University of Texas MD Anderson Cancer Center, Smithville, TX78957.

Tumor-promoting phorbol esters and insulin have similar effects on Reuber H35 rat hepatoma cell proliferation, including increased ornithine decarboxylase (ODC) enzyme activity, mitogenesis and stimulation of DNA synthesis. We investigated the relationship of ODC mRNA accumulation and phosphorylation of nuclear proteins in cells treated with either insulin or 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Both agents caused rapid accumulation of ODC mRNA, maximal 3 hr after treatment (4-5 fold greater than control cells) which returned to control levels within 18 hr. Simultaneous treatment with TPA and insulin leads to additive effects on ODC mRNA. Induction of ODC by TPA was blocked by down-regulation or inhibition of protein kinase C (PKC), consistent with a PKC-mediated mechanism. In contrast, PKC down-regulation had little effect on ODC induction by insulin. Although both agents stimulate ribosomal S6 protein phosphorylation, TPA also elicits rapid phosphorylation of specific nuclear proteins not observed with insulin. These results suggest that, although insulin and TPA share some common cytoplasmic signalling pathways, their effects on phosphorylation of nuclear proteins and transcription of ODC may be mediated by distinct factors. (Supported by NIH CA46629 to A.P.B.)

D 304 **NAB RECOGNITION OF H-RAS TRANSFORMED 10T1/2 CELLS, Donna A. Chow and David F. Tough, Manitoba Institute of Cell Biology, University of Manitoba, Wpg, Canada, R3E 0V9.** Natural antibody (NAb) recognition of early events in tumor development was assessed using ras/neo transfected 10T1/2 cells which exhibited a correlating range of ras mRNA expression and metastatic potential (Egan et al, Mol. Cell. Biol. 7:830, 1987). A G418-resistant, morphologically "normal" line which did not form metastasis and exhibited ras mRNA levels slightly less than the control 10T1/2 showed no change in NAb binding. The ras transfectant with the smallest increase in ras mRNA beyond the 10T1/2 bound almost twice as much NAb as 10T1/2 and further increases in ras mRNA were associated with a significant inversely correlating decrease in NAb binding. In addition, different plating density-dependent NAb binding patterns were demonstrated by the range of ras mRNA expressing cells with the intermediate expressors exhibiting consistent increases after growth from higher densities. Furthermore, infection of 10T1/2 with ras/neo increased the level and heterogeneity of NAb binding. Thus NAb may recognise changes in cell density and early stages of tumor development associated with small increases in H-ras expression, consistent with a role in tumor surveillance. Supported by the MRC and NCI of Canada.

D 305 PHORBOL ESTER INDUCTION OF RIBONUCLEOTIDE REDUCTASE IN BALB/c 3T3 MOUSE FIBROBLASTS, Bob K. Choy, Grant A. McClarty and Jim A. Wright, Department of Biochemistry, Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Canada R3E 0V9.

Ribonucleotide reductase is a rate limiting enzyme in the synthesis of deoxyribonucleotide precursors of DNA. Its catalytic activity requires both its large (M1) and small (M2) subunits which are non-coordinately expressed. While the M1 subunit is constituitively expressed by proliferating cells, the M2 subunit expression is cell-cycle dependant with a tight correlation to S-phase. The phorbol ester tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), has been shown to lead to altered expression of specific genes such as the proto-oncogenes *c-fos*, *c-myb* and *c-myc* as well as ornithine decarboxylase (ODC). We show TPA also strongly induces ribonucleotide reductase activity in proliferating BALB/c 3T3 fibroblasts. The enzyme activity is transiently elevated within ½ hour of treatment with 0.1μM of TPA and returns to near normal levels within 24-48 hours. Northern and Western blot analysis shows the induction of activity is due to an increase in M2 protein as a result of an 18-fold increase in the M2 mRNA levels at ½ hour and declines to normal levels by 48 hours. Contrary, the M1 subunit message and protein levels remain essentially unchanged with TPA treatment. Whether the elevation of M2 mRNA is due to either an increase in transcription or message stabilization, or both remains to be determined. As a positive control, ODC mRNA induction was analysed and we found it to be transiently elevated with similiar kinetics as others have reported. This work is supported by the National Cancer Institute of Canada.

D 306 THE ROLE OF PRISTANE AS A PROMOTOR OF 3-METHYLCHOLANTHRENE INDUCED LYMPHOID MALIGNANCIES IN RATS, Marvin A. Cuchens and Lenora R. Garrett, Dept. of Micro., Univ. of MS Medical Center, Jackson, MS 39216
Based on our previous observation that the incidence of 3-methylcholanthrene-induced lymphoid malignancies was significantly higher in pristane (2,6,10,14-tetramethylpentadecane) treated rats than in unprimed rats, studies to address the possible role of pristane as a tumor promoter were conducted. Studies to examine the effects of pristane on lymphoid cells from treated rats have demonstrated that pristane 1) is cell-associated and localized predominately within the plasma membranes, 2) elicits transient changes in membrane fluidity, and 3) concomitantly induces a change in the expression of histone proteins as well as chromatin conformation. In vitro studies were also conducted to compare the effects of pristane with 12-O-tetradecanoylphorbol 13-acetate (TPA), a known tumor promoter, on gene activation. Using established cell lines which were transfected with the pSV2cat plasmid and subsequently assayed for chloramphenicol acetyltransferase (cat) activity, it was demonstrated that pristane, as well as TPA, elicits activation of the cat gene in a dose dependent fashion. Collectively our results indicate that pristane may elicit cellular responses which are similar to the well characterized tumor promoter, TPA. Supported by PHS grant # R01 CA33111-04.

D 307 MODULATION OF HPV-18 AND BPV-1 TRANSCRIPTION BY SIMIAN VIRUS 40 LARGE T ANTIGEN, Michel Darmon, Catherine Bailly, Marie-Cecile Lenoir, and Bruno A. Bernard. Cell Biology Department, Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, 06565 Valbonne, France.

We have analysed the transcription from HPV-18 and BPV-1 promoters in cultured human keratinocytes, and its modulation by viral gene products. The transcription from the long control region (LCR) of HPV18 cloned in enhancer-promoter configuration ahead of the bacterial CAT gene is either inhibited by BPV-1 or HPV-18 E2 gene products but is stimulated by the SV 40 large T antigen in a dose-dependent manner. Using CAT constructs containing subfragments of the HPV-18 LCR cloned in enhancer configuration ahead of an enhancerless TkCAT plasmid, we show that the activation by SV 40 large T antigen is maximal (x 10) with the 230 bp RsaI-RsaI fragment, previously identified as a constitutive enhancer. This fragment does not contain any E2 binding sequence but contains an AP1 binding element.On the other hand, in the case of the BPV-1 E2 gene product, the SV 40 large T antigen can activate transcription from the BPV-1 LCR. Both factors can act synergistically, even when the BPV-1 E2 gene product is an otherwise inactive truncated form.

D 308 AN IN VITRO MODEL FOR THE DIFFERENTIATION OF HUMAN COLON CARCINOMA CELL LINES VIA STIMULATION OF THE SIGNAL TRANSDUCTION PATHWAY, Elisa Eiseman[1], Michael J. Kornstein[2], and Eric H. Westin[3], Departments of Human Genetics[1], Pathology[2], and Medicine[3], Medical College of Virginia, Richmond, VA 23298.
Calcium ionophore A23187 and phorbol-12,13-dibutyrate (PDBu) stimulate cellular responses, such as differentiation, through interaction with specific components of the signal transduction pathway. Treatment of three colon carcinoma cell lines, COLO 205, HT29, and SW480, with A23187 plus PDBu results in an irreversible decrease in proliferation as measured by uptake of ^{3}H-thymidine. Morphological changes associated with differentiation can be seen with Papanicolaou staining. Immunoperoxidase assays show that this differentiation process is also associated with increased expression of carcinoembryonic antigen (CEA) and decreased expression of KI-67, a proliferation antigen. This parallels normal colonic epithelium where CEA is expressed by mature, differentiated, non-proliferating cells, and KI-67 is expressed by immature, proliferating cells. Northern analysis of RNA from COLO 205 and HT29 shows that the increased expression of CEA seen in the immunoperoxidase assays correlates with an increase in the level of CEA mRNA. Concomitantly, there is a decrease in the level of c-myb and c-myc mRNA, genes which are frequently expressed in proliferating cells. The irreversible withdrawal of cells from the cell cycle in addition to the changes in morphology, cell surface markers, and gene expression indicate that these cell lines become terminally differentiated after treatment with A23187 plus PDBu. This system may therefore provide a useful model for the analysis of cellular events associated with both differentiation and neoplastic transformation in the colon and the role of oncogenes in these processes.

D 309 REGULATION OF A TISSUE FACTOR-LIKE GENE AND PROCOAGULANT ACTIVITY IN MURINE FIBROBLASTS BY PEPTIDE GROWTH FACTORS, Chris L. French*, Gouri Ranganathan*, David N. Fass+, and Michael J. Getz*, Department of Biochemistry and Molecular Biology* and Section of Hematology Research+, Mayo Clinic and Mayo Foundation, Rochester, MN 55905.
Tissue factor (TF) is a cell-surface receptor for factor VII serine protease and appears largely responsible for initiating the protease cascade leading to blood coagulation. TF or TF-like procoagulant activity (PCA) has also been implicated in the initial formation of a fibrin matrix and subsequent establishment of a metastatic focus by a variety of tumor cells. In this study, we show that transcription of a mouse gene with significant similarity to that encoding human TF is rapidly and transiently induced by serum stimulation of AKR-2B fibroblasts. In a similar fashion, factor VII-dependent PCA was essentially nondetectable in serum-starved cells but was strongly induced following serum stimulation. Both TF-like gene transcription and factor VII-dependent PCA were found to be superinducible by inhibitors of protein synthesis. A preliminary survey of several purified growth factors indicated that transforming growth factor β (TGF-β) is a potent inducer of factor VII-dependent PCA. In contrast, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and insulin were weak inducers. These studies identify a novel response of fibroblast-type cells to TGF-β and may have implications for both normal tissue repair and fibroproliferative disease. (Supported by NIH grants CA33643, HL117430, and by the Mayo Foundation.)

D 310 MECHANISM OF ACTION OF NEW TUMOR PROMOTERS: THE OKADAIC ACID CLASS, Hirota Fujiki, Masami Suganuma, Hiroko Suguri, Shigeru Yoshizawa, Kanji Takagi, Toshihiro Sassa, Naoto Uda, Werner W. Richter and Takashi Sugimura*, National Cancer Center Research Institute, and *National Cancer Center, Tokyo 104, Japan
The members of the okadaic acid class (OAC) are okadaic acid, dinophysistoxin-1 (35-methyl-okadaic acid) and calyculin A. Their tumor promoting activities are as strong as those of TPA-type tumor promoters, such as TPA, teleocidin and aplysiatoxin. However, tumor promoters of OAC did not bind to phorbol ester receptors in cell membranes, nor activate protein kinase C in vitro. These results indicated that a potent tumor promoting activity of OAC can be achieved by a different pathway other than activation of protein kinase C. Recently we demonstrated that ^{3}H-okadaic acid bound specifically to the particulate fraction and the cytosolic fraction of mouse skin. The specific binding of ^{3}H-okadaic acid to the particulate fraction was not inhibited by TPA-type tumor promoters. Moreover, unlabeled okadaic acid, dinophysistoxin-1 and calyculin A inhibited the specific binding of ^{3}H-okadaic acid to the particulate fraction of mouse skin dose-dependently, whereas okadaic acid tetramethyl ether, an inactive compound, did not. These results suggested that tumor promoters of OAC bind to their own binding protein(s) (receptors), present in mouse tissue, such as skin and brain. A protein kinase which was specifically activated by tumor promoters of OAC in vitro, was found in a cytosolic fraction of mouse skin and brain. Furthermore, the protein kinase activity and specific binding of ^{3}H-okadaic acid were found in the same fractions of DEAE-cellulose chromatography. In summary, the activation of this new protein kinase by tumor promoters of OAC might be a new pathway of tumor promotion.

D 311 MALIGNANT CONVERSION OF MURINE PAPILLOMA CELL LINES BY TRANSFECTION WITH fos BUT NOT E1A OR myc ONCOGENES. David A. Greenhalgh and Stuart H Yuspa, LCCTP, NCI, Bethesda, MD 20892 Murine cell lines 308 and SP-1 produce papillomas when grafted to nude mice and contain an activated Ha-ras oncogene consistent with their derivation from dimethylbenz[a]- anthracene treated skin. To investigate malignant conversion, these lines have been cotransfected with a murine plasmacytoma c-myc, adenovirus 5 E1A, FBJ vfos and a human c-fos/FBJ v-fos chimera together with pSV2neo as a selectable marker.Southern and Northern analysis confirmed the uptake and expression of exogenous oncogene DNAs. The cell lines were tested for malignant conversion by grafting and subcutaneous injection into nude mice. E1A, myc and neo control transfectants produced papillomas on grafting and no subcutaneous lesions. Grafts and subcutaneous injection of transfectants from both fos constructs where squamous cell carcinomas. By immunofluorescence E1A, myc and parental cell line tumors displayed an identical pattern of keratin expression, while fos tranfected tumors lost expression of the K1(67kD) consistent with their malignant phenotype. fos transfectants were unable to grow in agar and the tumors were negative for gammaglutamyl transferase activity. To show a direct cooperation between fos and ras ongoing studies include co-infection of primary murine keratinocytes with replication defective retroviruses. Since the fos gene product is a transcriptional enhancer with a specificity similar to AP-1, expression of AP-1 modulated genes is being assessed in tumors and cell lines to elucidate the fos coversion mechanism.

D 312 CONVERSION OF NEOPLASTIC REGRESSOR CELLS TO PROGRESSOR CELLS BY HOST REACTIVE CELLS, Jun-ichi Hamada, Noritoshi Takeichi, Futoshi Okada, Masuo Hosokawa and Hiroshi Kobayashi, Lab. of Pathol., Cancer Inst., Hokkaido Univ. Sch. of Med., Sapporo, 060 Japan

A clonal regressor cell line, ER-1 which was derived from a rat mammary carcinoma (c-SST-2) had very low tumorigenic properties, when s.c. injected into syngeneic rats. However, ER-1 cells induced a high incidence of tumor take at a low cell dosage when s.c. implanted after having first been attached to plastic plates (10x5x1mm). Furthermore, the tumors arising from the plate-attached ER-1 cells developed a much higher tumorigenicity and metastatic capacity. These acquired malignant properties remained stable for at least 6 months. In order to examine whether host cells reactive to plates play a role in the acquirement of malignant properties, ER-1 cells were cocultured with reactive cells obtained from tissues surrounding the plates. This conversion of ER-1 cells from a regressor type to a progressor type was induced by their cocultivation with host reactive cells. Culture supernatants taken from host reactive cells contained prostaglandin E_2, chemotactic factors to ER-1 cells and factors which enhanced the anchorage-independent growth of ER-1 cells. These findings suggest that those host cells which react to the plates may produce known or as yet unknown factors which directly and/or indirectly enhance the progression of regressor cells.

D 313 EXPRESSION OF INSULIN-LIKE GROWTH FACTOR II (IGF-II) IN MYOGENIC AND TROPHOBLAST TUMORS. H. Herbst, M. Hummel, G.Niedobitek, R. Schwarting, H. Stein. Institute of Pathology, Klinikum Steglitz, Free University Berlin, Hindenburgdamm 30, 1000 Berlin 45, FRG.

The insulin-like growth factor II (IGF-II) is variably expressed in developing and malignant tissues and may be involved in autocrine regulation of tumor growth. We have synthesized a gene encoding the human IGF-II polypeptide and expressed it in E.coli as a fusion protein with part of the trpE polypeptide. Purified IGF-II fusion protein was employed for the production of monoclonal antibodies specific for IGF-II and suitable for immunohistochemistry. The specificity was established by an ELISA with other human growth factors (IGF-I, PDGF-B, TGF-α). At present, we have focused on establishing the staining pattern for myogenic and trophoblastic human and rat tissues. The monoclonal antibody 5A6 detects developing skeletal muscle in rat embryos, indicating a cross-reactivity with the rat equivalent of IGF-II, multiplication-stimulating activity (MSA). In adult human muscle tissues, weak reactivity was variably observed with smooth muscle, while striated and heart muscle were negative. Leiomyomas showed a variable, usually slightly increased reactivity as compared to the surrounding muscle. In rhabdomyosarcomas, strong staining was localized to large cells, indicating that IGF-II expression is predominantly restricted to certain differentiation stages of striated muscle. Strong expression was observed in placental trophoblast and trophoblast-related tumors such as metastatic and primary chorioncarcinomas. IGF-II may therefore prove valuable as a serological and immunohistological marker marker for certain tumors.

D 314 THE STATE OF CELLULAR DIFFERENTIATION DETERMINES THE ACTIVITY OF THE ADENOVIRUS E1A ENHANCER ELEMENT. R.S. Herbst, M. Pelletier, and L.E. Babiss, The Rockefeller University, New York, N.Y. 10021

Mutants of type 5 adenovirus that lack all or part of the E1A enhancer element are still capable of transforming rat embryo fibroblast (CREF) cells at frequencies similar to or exceeding that produced by wild-type viruses. When analyzed for E1A gene expression in CREF cells, these enhancer mutant viruses and wild-type virus transcribe and accumulate E1A mRNAs at the same low constitutive rate. This contrasts with the results obtained using either differentiated or dedifferentiated rodent hepatoma cells, myeloma cells, or human Hela cells, where the absence of the E1A enhancer greatly reduces the rate of E1A gene transcription. These observations therefore suggest that E1A expression in undifferentiated cells is enhancer-independent. An in vitro analysis (including gel-mobility shift, exoIII stop, and DNase I footprinting analyses) of the cellular factors that interact with and modulate the activity of the E1A enhancer from all of the cell types, revealed similar DNA-binding activities for the E2f, ATF, EF-A, and sp1 proteins. However, an AP-3-like activity (TPA-inducible) which could be detected in all of the cell types, was at a 5-10 fold higher concentration in the undifferentiated rodent cells. Based on these observations, we propose a mechanism by which suppression of E1A expression by this nuclear protein might represent a crucial step in the establishment of the transformed cell phenotype.

D 315 Phenotypic Effects of Overexpression of PKCβ1 in Rat Liver Epithelial Cells L. L. Hsieh, S. Hoshina and I. B. Weinstein, Cancer Center and School of Public Health, Columbia University, New York, NY 10032.

We have used a previously described retroviral expression vector pMV7-PKCβ1 (Housey et al., Cell 52:343, 1988) to develop derivatives of two rat liver epithelial cell lines, K16 and K22, that stably express at least 10 fold higher PKC activity than control cells. Despite these high levels of PKC, these cells did not exhibit gross morphologic changes, anchorage independent growth or tumorgenicity. K16PKC-4 and K22PKC-2, two lines with the highest PKC enzyme activity, were studied further in terms of several responses to the phorbol ester tumor promoter TPA. When treated with 100 ng/ml of TPA, the control K16 and K22 cells displayed a slight change in morphology, whereas the K16PKC-4 and K22PKC-2 cells displayed a marked change in morphology. Northern blot analyses demonstrated that TPA induced increased levels of fos, myc, phorbin and ODC RNAs in control K16 and K22 cells, with maximum induction occurring at about 0.5, 1, 4 and 8 hours, respectively. In K16PKC-4 and K22PKC-2 cells, TPA induction of myc, phorbin and ODC RNAs was markedly enhanced, but this was not the case for fos RNA. In addition, the levels of myc RNA were constitutively higher in both the K16PKC-4 and K22PKC-2 cells, than in the control cells. Taken together, these results provide direct evidence that PKC plays a critical role in modulating the expression of myc, phorbin and ODC RNAs. On the other hand, overexpression of PKCβ1 is not itself sufficient to cause cell transformation. (Supported by NCI grant CA02656 to I. B. W.)

D 316 THE myb ONCOGENE PRODUCT IS A REGULATOR OF GENE EXPRESSION, Carlos E. Ibañez, Friedrich A. Grässer and Joseph S. Lipsick, Department of Pathology, M-012, UC San Diego, La Jolla, CA 92093-0612 and Research Division, Veteran's Administration Hospital, San Diego, CA 92161

The v-myb oncogene of avian myeloblastosis virus causes acute myelomonocytic leukemia in chickens and transforms cells of the myeloid lineage but not fibroblasts in vitro. Its product p48$^{v\text{-}myb}$ is a doubly truncated version of the normal cellular p75$^{c\text{-}myb}$. The function of p75$^{c\text{-}myb}$ and the mechanism by which p48$^{v\text{-}myb}$ is able to transform hematopoietic cells during early development is currently unknown. In order to study the functions of these proteins, our laboratory has developed an in vitro transformation assay for molecular clones of the v-myb oncogene (1,2). We have recently used this assay to identify specific domains of p48$^{v\text{-}myb}$ which are required for its nuclear transport, for its associated DNA-binding activity, for morphological transformation of myeloid cells, and for the growth of transformed cells in soft agar (3,4). We now demonstrate that p48$^{v\text{-}myb}$ and p75$^{c\text{-}myb}$ can function as transactivators of gene expression in cotransfection assays. In addition we have analyzed a series of deletion mutants of v-myb and thus far find that transactivation activity correlates with the capacity for morphological transformation.

1. Lipsick,J.S., C.E. Ibanez, and M.A. Baluda. J. Virol. 59:267-275.
2. Lipsick,J.S. and C.E. Ibanez. 1987. J. Virol. 61: 933-936.
3. Ibanez,C.E. and J.S. Lipsick. 1988. J. Virol.62: 1981-1988
4. Ibanez,C.E.. A. Garcia, U. Stober-Grässer, and J.S. Lipsick. 1988. J.Virol., in press.

D 317 **LOSS OF SYNDECAN** (cell surface proteoglycan) **EXPRESSION IN MOUSE MAMMARY EPITHELIAL CELLS AFTER TRANSFORMATION WITH A POINT-MUTATED c-Ha-*ras* PROTO-ONCOGENE.** Markku Jalkanen*, Jarkko Kirjavainen*, Scott Saunders**, Nancy Hynes*** and Merton Bernfield**, Medical Biochemistry*, University of Turku, SF-20520 Turku, Finland, Pediatrics**, Stanford University School of Medicine, CA 94305, USA and Ludwig Institute for Cancer Research***, Inselspital, 3010 Bern, Switzerland.

Syndecan is a heparan sulfate-rich proteoglycan, which binds epithelial cytoskeleton to interstitial molecules. Its expression during organ development follows morphogenetic rather than histological boundaries, suggesting that it may be one of the molecules at cell surfaces translating the extracellular matrix (ECM) influence into epithelial behavior. Transformation is known to alter cellular response to ECM, and therefore we have analyzed syndecan expression in mouse mammary epithelial cells transfected with a plasmid containing a glucocorticoid inducible MMTV-LTR linked to the point mutated c-Ha-ras EJ oncogene. In these cells the expression of 1.3 kb ras-mRNA was observed to be inducible within hours and to be high for weeks. At the same time the expression of syndecan mRNA (2.6 kb) decreased to less than one third of the original, demonstrating also no accumulation of syndecan mRNA observed for confluent normal epithelial cells. The reduction of syndecan mRNA-levels was evident before any observable morphological changes in the epithelial monolayers. Ras-induction also promoted these cells to form colonies in the soft agar. These anchorage-independent cells revealed also low expression of syndecan mRNA and low numbers of syndecan molecules on their surfaces. These findings, together with our previous data on spontaneously transformed cells (J. Cell Biol. 1987, 105 (4, Pt. 2) 132a), suggests that transformation reduces syndecan expression, and that this disappearance of syndecan can be one of the molecular events during carcinogenesis leading to abnormal behavior of malignant epithelial cells.

D 318 ANALYSIS OF THE c-myc AND K-ras PROTO-ONCOGENE IN THE CONVERSION OF NON-TUMORIGENIC HUMAN SQUAMOUS CELL CARCINOMA CELLS TO TUMORIGENICITY BY MMS TREATMENT. Ponnamma Kurian, Daniel Mannix, Fadia Dib-Hajj, Narinder Sital and George E. Milo, Department of Physiological Chemistry, The Ohio State University, Columbus, OH 43210

The non-tumorigenic human squamous cell carcinoma line, SCC-83-01-82, established from a tumor of the tongue can be transformed to a malignant phenotype by treatment with MMS (Hollering, etal., Second International Conference on Head and Neck Cancer, 1988). The MMS treated cells produced progressively growing tumors in splenectomized Balb/C nude mice, 40-50% of the time (5/11) within 3-4 months after subcutaneous injection. Cell lines established from these tumors have an increased tumorigenic vigor (100%) with only 5-7 days of in vivo latent period. In an attempt to study the mechanism of this malignant conversion, we analysed genomic DNA from the original cell line and the MMS-derived cell lines and tumors by Southern blot hybridization. Genomic DNA was digested with EcoRI, Hind III, PvuII, SstI or XbaI and then hybridized with a c-myc cDNA probe; no rearrangement in the c-myc gene was observed. Similarly, hybridization with v-K-ras probe after digestion with SstI did not indicate the presence of any activating point mutation in the 12th codon. These data suggest that c-myc rearrangement and/or mutation of K-ras at the 12th codon is not directly involved in the process of increased malignant vigor of SCC tumorigenesis.

D 319 **INDUCTION OF c-*MYC* AND c-*FOS* IN HAMSTER EPIDERMAL CELLS: BIPHASIC EXPRESSION INVOLVES PROTEIN KINASE C DURING EARLY EXPRESSION, BUT MOSTLY cAMP DURING LATE EXPRESSION.** Sujata Manam, Brian J. Ledwith, Matthews O. Bradley, and Warren W. Nichols, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

H5-MNNG, immortalized epidermal cells, expressed c-*fos* and c-*myc* as an immediate response to a variety of growth factors. c-*fos* induction by most of the growth factors tested was rapid and transient. Only serum induction persisted for up to four hours after stimulation. In contrast, c-*myc* exhibited a pronounced biphasic response to induction by serum, bombesin, vasopression and insulin. A strong, transient burst of expression at one hour after addition of these growth factors was followed by a subsequent burst of expression at 4-6 hours. EGF caused a broad peak of c-*myc* expression from one to four hours. FGFa, FGFb, and TGF-*β*, however, caused only a delayed, late induction of c-*myc*, with a peak at 6 hours. Phospholipid degradation induced by phospholipase C or the peptide melittin caused a rapid and transient burst of both c-*fos* and c-*myc* expression, as did Ca^{2+} ionophore A23187 and protein kinase C activators OAG and TPA. Dibutyiri-cAMP, however, caused a prolonged induction of both c-*fos* and c-*myc* expression from one to six hours after stimulation. These results suggest that phospholipid turnover, Ca^{2+}, and kinase C may be major components of a pathway leading to the rapid, early induction of both c-*fos* and c-*myc*; and that cAMP may be an important factor in the prolonged expression of c-*fos* by serum, and the late expression of c-*myc* during biphasic or delayed induction by a variety of growth factors.

D 320 USE OF SINGLE CELL ASSAYS TO DETERMINE THE BIOLOGICAL ACTIVITY OF THE FOS ONCOGENE PROTEIN. J. Meinkoth, M. Mulcahy, S. Fink, R. Goodman, R. Clark, F. McCormick and J. Feramisco. University of California, San Diego, La Jolla, CA., Tufts Medical School and Cetus Corp. Cellular protooncogenes comprise a highly regulated network of signals that govern normal cell growth. Mutations or altered levels of expression of any one of these critical signalling components can lead to carcinogenesis. We are studying the biological function of the fos protein(s) directly in single living cells using needle microinjection.to introduce functional fos protein(s) and anti-fos antibodies into the cell cytoplasm. Through this approach we hope to determine: 1) if the fos protein(s) is one component of a generalized transcriptional activation complex and 2) what other oncogene proteins are involved in the signal transduction pathway(s) that ultimately activate fos expression in the nucleus. To achieve this end, we are cloning the v-fos coding region into a baculoviral expression vector for microinjection and purifying a trp-fos fusion protein from a bacterial expression system for antibody production. As cell recipients for the microinjection studies, we are generating stable cell lines containing various enhancer elements cloned upstream from the bacterial β-galactosidase gene in order to analyze the effect(s) of fos protein expression on a cell-by-cell basis.

D 321 THE MOUSE MOS PROTO-ONCOGENE PRODUCT FUNCTIONS DURING OOGENESIS. Richard S. Paules[1], Roberto Buccione[2], Friedrich Propst[1], John J. Eppig[2], and George F. Vande Woude[1]; [1]BRI-BRP, NCI-FCRF, Frederick, MD 21701, [2]Jackson Laboratory, Bar Harbor, ME 04609.
c-mos proto-oncogene RNA expression is developmentally regulated in a tissue-specific manner and abnormal or constitutive expression in certain cell types can result in neoplastic transformation. The highest levels of mos mRNA are found in male and female germ cells. We examined metabolically labeled mouse oocytes in various stages of oogenesis for the presence of mos protein. Three different mos-specific antisera recognize a 39 kilodalton (kD) protein identical in size to the c-mos^{mu} protein expressed in transformed NIH/3T3 cells. We conclude that $p39^{mos}$ is the mouse c-mos proto-oncogene product. $p39^{mos}$ was detected in protein extracts from fully grown oocytes and oocytes undergoing meiotic maturation.

Microinjection of synthetic antisense oligonucleotides to c-mos^{mu} mRNA into fully grown oocytes resulted in the block of subsequent meiotic maturation steps, while sense c-mos^{mu} oligonucleotides had no significant effect. Specifically, oocytes microinjected with antisense c-mos^{mu} oligonucleotides progressed through germinal vesicle breakdown (GVBD) but failed to produce the first polar body. Cytogenetic analysis indicated that they were arrested at metaphase I. Thus, the mos protein product is present during oocyte maturation and is necessary for completion of meiosis. These results are similar but not identical to mos function in Xenopus (Sagata et al., Nature, 335:519-525, 1988) where the product is believed to be involved in the activation of maturation promoting factor.
Research sponsored in part by the NCI, DHHS, under contract NO. NO1-CO-74101 with BRI.

D 322 THE HORMONE BINDING DOMAIN OF STEROID RECEPTORS CAN CONFER HORMONE REGULATION UPON NUCLEAR ONCOGENES, Didier Picard and Keith R. Yamamoto, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143-0448. The glucocorticoid and estrogen receptors are signal transducers that interact both with the signal and with their target genes. We have recently defined a novel protein repression function for the steroid binding domain. We have proposed that the heat shock protein hsp90, which binds selectively to the unliganded receptor, mediates the hormone-reversible inhibitory function which can affect several activities resident on the same polypeptide without strict regard to structure. Indeed, hormone regulation can even be conferred upon heterologous proteins. We have fused the steroid binding domains of the glucocorticoid and estrogen receptors to the adenovirus E1A protein and to the cellular protooncogene c-Fos and its oncogenic counterpart v-Fos. We show that the transcriptional regulatory and transforming functions thereby become regulable at the protein level. We are presently exploiting these fusion proteins, which can be turned on and possibly off with fast kinetics, to study the mode of action of these oncogenes.

D 323 ALTERATIONS IN EGF-DEPENDENT PROMOTER ACTIVITY INDUCED BY TRANSFORMING GROWTH FACTOR TYPE β, Gouri Ranganathan and Michael J. Getz, Department of Biochemistry and Molecular Biology, Mayo Clinic and Mayo Foundation, Rochester, MN 55905.
Transforming growth factor type β (TGF-β) is a pleiotropic regulator of cell growth and differentiation which can potentiate or otherwise modify cellular responses to different growth factors such as epidermal growth factor (EGF). Since cellular responses to peptide growth factors are mediated, in part, through the regulation of specific gene expression, we have studied the effects of TGF-β on the EGF-dependent transcription of a diverse panel of genes including the fibronectin gene, the cytoskeletal β- and γ-actin genes, and the c-fos proto-oncogene. The addition of a combination of TGF-β (10 ng/ml) and EGF (10 ng/ml) to quiescent AKR-2B cells maintained in serum-free medium resulted in a strong synergistic stimulation of transcription which was not evident using either growth factor individually at concentrations of 20-100 ng/ml. This effect was evident within 10 min and did not require the continual presence of TGF-β in the culture medium. Increased responsiveness of some, but not all promoters to EGF, was evident for up to 6 hrs following a brief 10 min exposure to TGF-β. Similar exposure to either platelet-derived growth factor (PDGF) or fibroblast growth factor (FGF) had little or no effect on EGF-dependent transcription. These results suggest that the transcriptional response of at least some specific genes to EGF can be stably altered as a consequence of exposure to TGF-β. (Supported by NIH grant CA33643 and by the Eagles Cancer Research Fund.)

D 324 IDENTIFICATION OF UV INDUCIBLE PROTEINS WHICH RECOGNIZE A TGACAAGA SEQUENCE. Zeev A. Ronai, Grace Lee, Ester Okin and I.Bernard Weinstein, Comprehensive Cancer Center and Institute of Cancer Research, Columbia University, New York, NY 10032.
The exposure of mammalian cells to UV light or other DNA damaging agents triggers several reaponses which are presumably involved in cellular defence and recovery. We have recently identified a UV-inducible factor(s) that acts in trans to initiate asynchronous replication of integrated polyoma DNA sequences in rat fibroblasts (J. Virol.62: 1057, 1988). In the present study we employed a 257 base pair polyoma virus MboII-HpaII restriction enzyme fragment, that contains the origin of replication and regulatory region of this virus, to further characterize these factors. By analyzing protein complexes formed with this DNA fragment using gel retardation and DNase I footprinting methods, we identified a set of DNA binding proteins that are induced in normal rat fibroblasts at 6-24 hours after UV exposure. These proteins bind to a specific octamer TGACAAGA sequence in polyoma DNA (position 5255-5262) which we have designated the "UV response element" (UVRE). Employing a 12 mer duplex oligonucleotide that contains the UVRE sequence and a UV cross-linking method we further identified a set of UV-inducible proteins that specifically bind to the UVRE. These proteins are about 34, 39, 54, 58, and 78 Kd in size. We suggest that these proteins may play a critical role in mediating various cellular responses to DNA damage in mammalian cells.

D 325 CONTROL OF A HUMAN DIFFERENTIATION-SPECIFIC KERATIN GENE IN TRANSGENIC MOUSE KERATINOCYTES. D. Rosenthal, P. Steinert, S. Chung, S. Yuspa, and D. Roop. NCI, Bethesda, MD 20892 In the epidermis, morphological differentiation as well as malignant transformation closely correlates with a distinct pattern of keratin gene expression. The major keratins produced in basal cells have been designated K5 and K14, whereas keratins K1 and K10 represent the major differentiation products of the maturing suprabasal keratinocytes. In multistage mouse skin carcinogenesis experiments, K1 and K10 have been shown to be suppressed in malignant tumors. In order to study the factors responsible for the expression of these genes, transgenic mice have been produced using the entire human K1 gene plus flanking sequences in a 12 kb insert. Primary cell cultures from newborn K1 transgenics were established in order to examine the effect of calcium and retinoids on human K1 expression. Elevating the levels of calcium in the medium resulted in the induction of both mouse and human K1. Pretreatment of cells with retinoids completely suppressed this induction of the mouse gene, while the human K1 gene was more resistant to this suppression. These results suggest that: 1) the human 12 kb insert contains all the necessary cis-acting elements to respond to the calcium signal, and 2) other cis-acting elements, not present within this insert, may function independently to regulate the response of K1 to retinoids. Since K1 is expressed differentially in benign and malignant tumors, this system may prove useful in elucidating the factors responsible for the modulation of expression during malignant conversion.

D 326 PATHOBIOLOGICAL EFFECTS OF ALKYL-GLYCEROLIPIDS ON HUMAN LUNG CANCER CELLS. Koji Sasajima, Masao Miyashita, Toshiaki Shirota, Eiji Uchida, Masahiko Onda and Curtis C. Harris, First Department of Surgery, Nippon Medical Shool, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113, Japan, and Laboratory of Human Carcinogenesis, NCI, Bethesda, MD 20892
It has been reported that alkyl-phospholipids inhibit growth and induce differentiation of malignant cells. In human lung cancer cells, the cytotoxic effects of certain anticancer agents relate to the activity of prostaglandin synthesis. In this study, we examined the effects of alkyl-glycerolipids (an inhibitor of phospholipase A_2, AGL) in human lung cancer cells in vitro and in vivo. When adenocarcinoma A549 cells were inoculated subcutaneously into nude mice, the cells formed tumors and underwent morphological differentiation spontaneously without AGL treatment. The undifferentiated cells developed glandular structures that contained mucin-producing signet ring cells. However, the untreated A549 cells did not undergo differentiation in vitro. AGL significantly inhibited growth of A549 cells, but caused little effects on differentiation of tumor cells in nude mice, when compared with untreated mice. AGL caused signet ring cell-like morphology in vitro. These results suggest that the metabolism of lipids may regulate growth and differentiation of human lung cancer cells.

D 327 REGULATION AND ROLE OF IFN GENE EXPRESSION DURING DIFFERENTIATION OF EMBRYONAL CARCINOMA CELLS, Skup,D., Haggarty,S., Paterno,G.D., Ponton,A., Daigneault,L.and Belhumeur,P. Institut du Cancer de Montréal, 1560 Sherbrooke East, Montréal, Québec, Canada.
Embryonal Carcinoma (EC) cells closely resemble undifferentiated cells of the early embryo. One of the characteristics shared between the two cell types is the inability to produce interferon. In the embryo, competence for IFN induction appears during development. EC cells made to differentiate in vitro rapidly become inducible by viruses and dsRNAs. We have investigated the mechanisms underlying this regulation and the possibility that formation of endogenous dsRNA is involved in the differentiation process through an autocrine production of IFN. A DNA-binding factor specific to EC cells which recognizes a sequence within the HuIFNβ promoter has been identified. We propose that this DNA binding factor is a repressor responsible for the lack of response of EC cells to induction. The DNAse footprint pattern of the HuIFNβ promoter in differentiated cells is very different from that seen in EC cells. In the former a large area of the promoter binds protein, whereas, in the latter only a short sequence is protected. The action of the putative repressor may thus be to prevent the interaction of proteins which are required for inducibility. We have also found that a naturally occuring pair of sense/anti-sense RNA exists in many cell types. These complementary RNA's do not form duplexes in EC cells. However, the appearance of dsRNA occurs during differentiation at times when the cells are already competent of IFN induction. The possibility that endogenously produced IFN is directly involved in differentiation is suggested by our observation that the efficiency of obtaining untransformed derivatives is greatly decreased by the presence of anti-IFN antibodies in the culture medium.

D 328 DIFFERENTIAL SCREENING REVEALS REDUCED TRANSCRIPTION OF A MITOCHONDRIAL GENE IN TUMOURS ASSOCIATED WITH 11p REARRANGEMENTS: WILMS' TUMOUR AND HEPATOBLASTOMA. M.H. Litte 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 on 11p. These sequences may act by either (i) downregulating 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 760bp with a poly A+ tail. Hybridization of this clone with mRNA from the original WT/kidney revealed a 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 is assumed to be under some transcriptional control from the nucleus. This study provides preliminary evidence for down-regulation of a non-nuclear gene in embryonal tumours associated with 11p rearrangements.

D 329 SYNTHESIS AND STABILITY OF C-MYC PROTEINS IN DIFFERENTIATING MURINE ERYTHROLEUKEMIA CELLS. Gerald D.Spotts, and Stephen R.Hann Dept. of Cell Biology, Vanderbilt Univ. Sch. of Med., Nashville, Tenn. 37232. Although expression of c-myc mRNA is associated with proliferation, during the differentiation of several cell lines expression of c-myc mRNA remains relatively high, suggesting that c-myc proteins may have a function in terminally differentiated cells. Furthermore, we recently reported that there are two c-myc proteins initiating at two distinct initiation codons (Hann, et al., Cell 52, 185-195). To investigate whether translational and post-translational regulation of c-myc occurs during the DMSO-induced differentiation of Friend virus-infected murine erythroleukemia (MEL) cells, we examined the synthesis, stability, and localization of these nuclear phosphoproteins. Analysis of c-myc protein synthesis revealed a direct correlation with c-myc mRNA expression. However, there were differences observed in protein stability. Pulse-chase and western analysis of differentiating cells demonstrates a stabilization of c-myc proteins within the first 24 hrs. In later time points, c-myc proteins were destabilized. The possibility of differential function for these two proteins is being further investigated by overexpressing c-myc 1 or 2 in MEL cells. The effects of culture conditions and growth factors on the stability and localization of these two proteins is also being studied.

D 330 UCN-1028C, A NOVEL MICROBIAL COMPOUND, IS A SPECIFIC INHIBITOR OF PROTEIN KINASE C, Tatsuya Tamaoki[1], Eiji Kobayashi[1], Hirofumi Nakano[1], Makoto Morimoto[2], [1]Tokyo Res. Lab., [2]Pharmaceutical Res. Lab., Kyowa Hakko Co., Tokyo 194, Japan,
Protein kinase C (PKC) plays crucial roles in signal transduction following receptor activity by hormones, in cellular proliferation and in differentiation. Therefore, A potent and specific inhibitor of PKC should be useful to understand and characterize the biochemical role of PKC. In the course of screening inhibitors of PKC, we have found staurosporine, the most potent inhibitor of protein kinases and UCN-01, a selective inhibitor of PKC. We have now isolated UCN-1028C, a specific and potent inhibitor of PKC (IC_{50},0.05μM), from culture of Cladosporium cladosporioides. Even as high as 50μM of UCN-1028C only slightly inhibited cAMP-dependent protein kinase. UCN-1028C could not inhibit protein kinase activity of the catalytic domain of PKC showing that the regulatory domain is the target for this inhibitor. In addition UCN-1028C showed inhibitory effects on the binding of ^{3}H-phorbol dibutyrate to PKC.

UCN-1028C showed strong cytotoxic effects against tumor cells, HeLa S3 (IC_{50},0.18μg/ml) and MCF-7 (0.14μg/ml). Interestingly, UCN-1028C showed antitumor activity against murine lymphocytic leukemia P388 in vivo. It is conceivable that the cytotoxic and antitumor activity of UCN-1028C is due to the specific inhibition of PKC.

Thus, UCN-1028C is the first specific inhibitor of PKC with high potency, and will contribute to the study of the role of PKC in various cellular functions.

D 331 EXPRESSION OF CELLULAR ONCOGENES IN HUMAN LYMPHOMA AND LEUCEMIA, Hans Tesch, Michael Collasius, Stefan Guder, Manfred Jücker, Volker Diehl, Hilmar Bading and Karin Moelling, I.Medizinische Klinik, Universität Köln, 5000 Köln 41, FRG and Max-Planck-Institut für Molekulare Genetik, 1000 Berlin 33, FRG

Cellular oncogenes are frequently activated or deregulated in human tumor cells. We have analysed the expression of a variety of cellular oncogenes in human lymphoma and leucemia cells. Northern blot experiments revealed that some oncogenes (c-myb,c-met, c-src2, p53) were highly expressed in certain lymphoma and leucemic cells, depending either on the differentiation stage or on so far unknown activation mechanisms. Chromosomal in-situ hybridization analyses demonstrated that the c-met oncogene is close to the breakpoint t(7;21) (q22; p12) in a Hodgkin cell line. Aberrant c-fes specific transcripts were found in three independent lymphoma cell lines. High levels of c-myb mRNA were detected in myeloid and T cell tumors. Using monoclonal antibodies against the c-myb product, the protein was analysed in these cells by immunofluorescent staining, flow cytometry and Western blot experiments. The results demonstrate differential expression of c-myb mRNA and protein at the single cell level.

D 332 CONSTITUTIVE EXPRESSION OF NMYC BLOCKS RETINOIC ACID (RA) INDUCED DIFFERENTIATION BUT NOT GROWTH ARREST OF HUMAN NEUROBLASTOMA (NB) CELL LINES. C.J. Thiele, D. Feltner, S. Breit*, M. Schwab*, and M.A. Israel. Mol. Gen. Sect., Ped. Branch, NCI, and *DKFZ, Heidelberg, FDR. Spontaneous and chemotherapy induced maturation of NB tumors has been observed in vivo and NB cell lines can be induced to differentiate in vitro suggesting that NB tumors are blocked in their ability to differentiate and arrest cellular growth. Since 20% of NB tumors contain amplification of the NMYC gene and this genetic alteration is associated with rapid tumor progression and a poor clinical response, we were interested in the effects of constitutive expression of NMYC on the ability of NB to differentiate. A plasmid containing the 3'portion of exon 1 and exons 2 and 3 of NMYC(trans-NMYC) and a G418 selectable marker was transfected into the NMYC amplified NB cell line SMS-KCNR. There was no difference in the relative growth rates between control (53B) and NMYC transfected cell lines constitutively expressing transfected NMYC (422 and 512) or not (411). RA treatment of cells resulted in a marked decrease in ^{3}H-Thy. incorporation in all cell lines suggesting NMYC does not affect RA-induced arrest of cell growth. However, the cell lines expressing trans-NMYC failed to morphologically differentiate as measured by neurite extension. Northern analysis revealed decreased expression of 68KD neurofilament and GAP43 mRNA in the 422 and 512 NB cells which fail to differentiate. However, RA induced regulation of MYB expression was not affected by the constitutive expression of NMYC in RA-512 cells. RNAse protection analysis indicates that expression of the endogenous NMYC gene is down-regulated, while expression of trans-NMYC remains high during RA treatment indicating that high levels of NMYC mRNA can block NB differentiation. Unlike other systems in which constitutive expression of MYC blocks differentiation and arrest of cell growth, constitutive expression of NMYC inhibits RA-induced differentiation of NB cells but has no affect on RA-induced arrest of cell growth.

D 333 REGULATION OF THE PHORBOL ESTER-RESPONSIVE PROMOTER FOR CATHEPSIN L, A TRANSFORMATION-SENSITIVE PROTEASE THAT IS SECRETED, Bruce R. Troen and Michael M. Gottesman, Laboratory of Molecular Biology, NCI, NIH, Bethesda, MD 20892

The major excreted protein (MEP) of mouse fibroblasts is the precursor to a lysosomal acid protease (cathepsin L) induced by malignant transformation, growth factors, and tumor promoters. We have previously cloned a fully functional gene for MEP from NIH 3T3 cells that is an active cathepsin which is secreted, glycosylated, and processed intracellularly as in wild-type NIH 3T3 cells. When subcloned into chloramphenicol acetyl transferase (CAT) expression vectors, the promoter region for the MEP gene exhibits regulated expression in transfected cells. Both 4 kb and 345 bp fragments in the 5'-flanking region of the MEP gene confer CAT activity that is stimulated by cAMP treatment, but is paradoxically inhibited by phorbol ester treatment. The smaller fragment appears to confer greater constitutive promoter activity. Primer extension experiments were performed using RNA from cells transiently transfected with either a CAT construct with the 4 kb 5'-flanking region or a construct that contains additional sequences that include the first two exons and introns and part of the third exon of the MEP gene. Phorbol ester treatment increases the amount of the transcript from the construct containing introns, whereas levels of the transcript from the construct containing only the upstream sequences are reduced by phorbol ester treatment. The sequence of the MEP gene upstream from the transcriptional initiation site contains two potential binding sites for the AP-2 transcriptional factor, which has been shown to mediate both phorbol ester and cAMP responsiveness of other promoters. These results suggest that the MEP gene is regulated in a complex manner by these upstream sequences in concert with elements in either the first or second intron. We are further investigating the regulation of the MEP promoter by constructing deletion mutants to define the role of positive and negative regulatory elements in the upstream and intron regions.

D 334 BENZO(A)PYRENE DIOLEPOXIDE-DNA ADDUCTS IN HUMAN LYMPHOCYTES, Kirsi Vähäkangas[1], Erkki Yrjänheikki[2] and Olavi Pelkonen[1], Department of Pharmacology and Toxicology, University of Oulu[1], and Institute of Occupational Health[2], Oulu, Finland.

Human lymphocytes are able to metabolize benzo(a)pyrene (BP), as indicated by the existent AHH-activity and the capability to activate BP to DNA binding species. Because blood is one of the few tissues that can be taken from healthy persons, blood lymphocytes are studied as possible indicators of biologically meaningful carcinogen exposure. In addition to the need for very sensitive methods for the BP-DNA adduct measurements after environmental exposure, it is also important to study the stability of the adducts in human lymphocyte DNA and to study to what extent there is individual variation in the activation, and whether this is constant by time. We study these various aspects both in vivo and in vitro with human lymphocytes. We are currently following up a material of about 160 coke oven workers, whose lymphocyte DNA was first studied before the coke oven started its function. The second set of samples were taken 6 months after the start. The preliminary results indicate that there are no adducts by synchronous fluorescence spectrophotometry (SFS) due to smoking, and that there is increase in the amount of adducts after the first 6 months of working in the coke oven. Cultured, PHA-stimulated human lymphocytes activate BP to DNA-adducts measurable by SFS. There is a large interindividual variation in the amount of adducts, and the intraindividual variation in the activation capability seems to be rather small.

D 335 DIFFERENTIAL EXPRESSION OF THE 7B2 GENE IN HUMAN LUNG CARCINOMA CELLS, W.J.M. van de Ven, A.J.M. Roebroek, A. van Bokhoven, G.J.M. Martens*. Department of Biochemistry and Department of Zoology*, University of Nijmegen, Nijmegen, The Netherlands.
A human pituitary cDNA clone with an open reading frame for a 21 KD protein was isolated. Computer analysis of the deduced amino acid sequence revealed that it contained the complete structure of the 7B2 polypeptide, a highly conserved protein whose function is not known at present. Furthermore, a serine phosphorylation consensus sequence appeared present and also domains resembling the GTP binding domains characteristic for GTP binding proteins. The gene encoding 7B2 is a single copy gene which could be mapped by in situ hybridization to region q13 of chromosome 15. Northern blot analysis indicated that the major transcript of 7B2 measures 1.3 kb in size. Expression of the gene seems restricted to neurons and endocrine cells. We have also studied the expression of 7B2 in human lung carcinomas. Expression of the 7B2 gene could hardly be detected by Northern blot analysis in primary human adenocarcinomas or squamous cell carcinomas (15 cases tested) and also not in cell lines derived from these (3 cell lines tested). In primary small cell lung carcinomas (SCLCs), expression levels of 7B2 appeared variable. Of eight cases tested, 4 exhibited a high level of expression, 2 a moderate level and 2 were negative. In SCLC cell lines, however, a clear distinction could be made between SCLC of the variant type (SCLC-V) and of the classic type (SCLC-C). In none of the 6 SCLC-Vs tested, 7B2 transcripts could be detected. All SCLC-Cs (9 cell lines tested), however, exhibited high levels of 7B2 expression. Finally, carcinoids expressed very high levels of 7B2 mRNA. These data indicate that the 7B2 gene could be a useful marker to differentiate between human lung epithelial cell types.

D 336 GROWTH REGULATION OF CANCER CELLS THROUGH GAP-JUNCTIONAL INTERCELLULAR COMMUNICATION, Hiroshi Yamasaki, D.Jim Fitzgerald, Fumitaka Katoh and Marc Mesnil, Programme of Multistage Carcinogenesis, International Agency for Research on Cancer, Lyon, France.
Gap-junctional intercellular communication (GJIC) is believed to play an essential role in maintaining homeostasis by evening the intracellular level of growth control factors among neighbouring cells. We propose that one of the mechanisms of loss in growth control of cancer cells is their loss of GJIC with surrounding normal cells. We indeed found altered GJIC and/or gap-junction gene expression in certain transformed and tumor cells. Rat liver epithelial cell lines, for example, showed progressive reduction in communication capacity as they showed higher degree of transformed phenotypes. Similarly, analysis of primary hepatocellular carcinomas of rats showed that the level of mRNA which codes for major gap-junction protein was drastically reduced. On the other hand, transformation of fibroblasts (3T3 cells) by chemicals, radiation or various oncogenes did not result in reduced communication. However, such transformed cells did not communicate with surrounding normal cells. When communication between transformed and non-transformed cells was induced, transformed cells do not form distinct foci over normal monolayer cells. These results suggest that transformed (tumor) cells acquire reduced ability in GJIC with surrounding normal cells by two different ways: (1) reduced GJIC among transformed cells and (2) communication inhibition between transformed and non-transformed cells (selective communication). Such alterations may play an important role in the uncontrolled growth of cancer cells and reversal of these alterations may be one mechanism of tumor suppression.

D 337 CLONING AND CHARACTERIZATION OF A HUMAN EPITHELIAL CELL SPECIFIC mRNA LOST DURING MAMMARY CARCINOGENESIS, Paul Yaswen, Douglas Trask, Deborah Zajchowski, Donna Peehl, Martha Stampfer, and Ruth Sager, Lawrence Berkeley Laboratory, Berkeley, CA 94720, Dana-Farber Cancer Institute, Boston, MA, and Stanford University School of Medicine, Palo Alto, CA

Using subtractive hybridization to search for specific genes whose decrease in expression may be relevant to mammary carcinogenesis, we have identified an mRNA whose steady state expression is decreased more than 50-fold in human mammary epithelial cells transformed in vitro with benzo(a)pyrene plus Kirsten sarcoma virus. This 1.7 kb mRNA, NBS-1, is stably expressed in normal human mammary and prostatic epithelial cells. It is absent in normal human skin and bronchial epithelial cells, as well as tumor cell lines of breast and other tissue origins. The cDNA sequence contains a 671 base pair open reading frame preceded by an extremely long (>500 bp) 5' non-coding region. The NBS-1 transcript shares limited 5' sequence similarity with at least two more ubiquitously expressed mRNAs. NBS-1 may be a marker of epithelial differentiation and may also play a role in tumor suppression.

D 338 **REVERSIBLE AND IRREVERSIBLE TUMOR PROGRESSION FROM REGRESSOR TUMOR,** H. Kobayashi, J. Hamada, F. Okada, J. Ren, J. Hasegawa, N. Takeichi and M. Hosokawa, Lab. of Path., Cancer Inst. Hokkaido University School of Medicine, Sapporo, Hokkaido 060 Japan Since there are few experimental models of tumor pro- gression, we have used two lines of regressor tumors and tried to change these regressor clone cells to more malignant progressors by certain progression enhancing-factors such as plastic plate, spongel and quercetin. QR32 clone, fibrosarcoma of C57BL mice regresses in the normal host after 10^6 cells are transplanted sc. ER-1 clone, mammary carcinoma of SHR rat does the same. When these regressor cells are attached to a plastic plate, or to spongel, or exposed to host-reactive cells, or to quercetin and transplanted sc, these regressor cells are converted to malignant progressors in which only 500 - 1,000 cells are enough to kill the host with invasion and metastasis. More interestingly, 10 fold of PGE-2 were produced from the progressor tumor converted from QR 32; the microvilli appear abundantly on the surface of both of the above lines of progressor cells. Both PGE-2 production and microvilli seem to be good indicators of in vivo growth potential and progression of tumors. Progression's state is stable and irreversible, with chromosomal abnor- malitites, at least 6 months after conversion to progres- sors from regressors by plastic plate, spongel and host-reactive cells, but it is unstable and reversible to the regressor only within one month when the progressor is induced by quercetin. Regarding mechanisms of the irreversible progression, both selection (clonal evolution) and real conversion to progressors are possiblities, but the latter seems more plausible. From the above findings, it is assumed that certain regressor tumors may be useful as a model for the study of tumor progression.

Tumor Biology

D 400 The Fibroblast Response to Anoxia: A Potential Direct Role in Wound Healing and Malignant Conversion. Garth R. Anderson, Daniel L. Stoler, and Lisa A. Scarcello, Department of Molecular and Cellular Biology, Roswell Park, Buffalo, NY 14263.

Although the role of oncogenes in neoplastic transformation is becoming clearer, the genetic basis of malignancy is obscure. Normal fibroblasts respond to anoxia in a process which if expressed in an uncontrolled fashion may underly fundamental aspects of malignancy.

Normal rat fibroblasts when subjected to anoxia exhibit a staged response. In the first stage (0-6 hr) retrotransposon VL30 RNA is induced. In the second stage (6-12 hr) DNA replication ceases, hexose transport increases, and six major intracellular proteins are induced. In the third stage (after 12 hours), three secretory proteins are induced including the protease cathepsin L and a p61 encoded by VL30 RNA. Not until much later (after 72 hours of anoxia) does cell viability begin to decline. Following return to aerobic conditions, a factor is secreted which induces a delayed contractile response.

These responses represent a close match to the needs of fibroblasts during their role in the early stages of wound healing. Here fibroblasts invade the wound, are metabolically active under essentially anoxic conditions, and participate in removal of wound debris. Malignant cancer cells exhibit certain of these characteristics regardless of oxygen availability. Foremost is the ability to penetrate and invade tissue boundaries, a process involving cathepsin L. The same pattern of metabolic reliance on glycolysis is widespread in malignant tumors. Particularly intriguing is the potential role of VL30 in these processes. This retrotransposon is expressed at high levels in many cancers, is induced in mouse fibroblasts by tumor promoters, and represents one of two cell-derived inserts in the Kirsten and Harvey sarcoma virus genomes.

D 401 **A Direct Role of Homing Receptors and Vascular Addressins in the Dissemination of Murine Lymphoid Metastasis,** R.F. Bargatze, P.R. Streeter, and E.C. Butcher. Deptartment of Pathology, Stanford University, Stanford, CA 94305

Normal lymphocytes being uniquely mobile cells throughout much of their lifecycle, it is reasonable to propose that the dissemination of malignant lymphocytes may reflect the expression of normal lymphocyte mobility and "homing" mechanisms. The migration and traffic of normal lymphocytes to lymph nodes is controlled in large part by the regulated expression of surface homing receptors for high endothelial venules (HEV); as well as expression of vascular addressins (endothelial cell binding determinants for lymphoid cells) expressed on the lumen of specialized venules that mediate the extravasation of circulating lymphocytes from the blood into lymphoid organs and sites of chronic inflammation. We have reported that lymphomas that bind well to HEV in an in vitro assay disseminate widely via the blood, involving all lymph node groups symmetrically. In contrast, although both HEV-binding and non-binding lymphomas gain access to the blood, non-binding lymphomas do not cause distant lymph node involvement. Involvement of lymph nodes by these lymphomas is limited to nodes draining the local tumor at the site of injection. Here we report direct in vivo and in vitro observations demonstrating that expression of functional homing receptors for HEV and vascular addressins act to play an important role in the dissemination of lymphomas and leukemias to HEV-bearing organs. (Supported by NIH grants AI-19957, CA-07879, AI-09072, ACS grant CA-S-67-87, and an award from the Veterans Adminstration.)

D 402 Genetic events contributing to malignant conversion of human skin keratinocytes transfected with the cellular Harvey-ras oncogene.
P. Boukamp[+], R.T. Dzarlieva[+], E.J. Stanbridge[++] and N.E. Fusenig[+]; [+]German Cancer Research Center, Heidelberg, FRG; [++]University of California Irvine, Irvine, Ca.
Transfection of spontaneously immortalized human skin keratinocytes (HaCaT) with the c-Ha-ras oncogene resulted in clones with different growth behaviour in vivo. Twelve clones with higher levels of ras integration and expression were tumorigenic but only 6 formed malignant squamous cell carcinomas. The others grew to benign cystic tumors with varying grades of dysplasia. Thus, ras expression was associated with tumor growth but malignant conversion obviously required additional genetic alterations. New chromosomal rearrangements were preferentially found in the malignant clones. Although similar alterations also occurred in later passage HaCaT cells without leading to malignancy, these genetic changes in recombination with the ras oncogene may well contribute to the malignant phenotype. Alternatively or in addition the integration site could be crucial and therefore chromosomes containing the integrated ras oncogene were transferred to untransfected, nontumorigenic HaCaT cells via micro-nuclei transfer. Preliminary results indicate that such a chromosome from a malignant clone was able to induce malignancy in the parental HaCaT cells, while after transfer of a chromosome from a nontumorigenic clone the recipient HaCaT cells remained nontumorigenic. Thus, to our knowledge this is the first example of malignant conversion of human epidermal cells by transfer of a single chromosome.

D 403 DEGRADATION OF EXTRACELLULAR MATRIX PROTEINS BY CATHEPSIN B, Michael R. Buck, David G. Karustis, Nancy A. Day, Kenneth V. Honn and Bonnie F. Sloane, Department of Pharmacology, Wayne State University, Detroit, MI

Cathepsin B has been hypothesized to play a role in the degradation of the extracellular matrix during tumor cell metastasis. We report a specific proteolysis of the extracellular matrix proteins fibronectin and type IV collagen at pH's 5.0 and 7.4. The cleavage of type IV collagen gave products distinct from those produced by latent type IV collagenase, occurred in the presence of 15 mM EDTA and was completely inhibited by the cysteine proteinase inhibitor E-64. Fibronectin was degraded into a number of high MW products (100-200 kD) which underwent further degradation with longer times of incubation, and, two smaller products of18 and 22 kD. Further analysis of the degradation of laminin by cathepsin B indicates that under non-reducing, non-denaturing conditions a single 70 kD fragment is released from the laminin molecule. We are currently determining the cleavage site that produces this fragment. We have carefully analyzed the pH profiles for the degradation of laminin, fibronectin and type IV collagen since cathepsin B has been shown to lose activity above pH 7.0. Our data indicates that cathepsin B undergoes autodegradation at neutral pH. This autodegradation can be inhibited by E-64. This is significant in that cathepsin B released from a tumor cell and exposed to a high concentration of protein substrates, or, cathepsin B bound to the plasma membrane and physically unable to degrade itself, might be capable of degrading the components of the extracellular matrix under pH conditions found extracellularly.

D 404 ANALYSIS OF THE RELATIONSHIP BETWEEN PRIMARY TUMOUR CELLS AND METASTATIC CELLS IN INVADED TUMOUR DRAINING LYMPH NODES, Ailsa Campbell, Philippa Whitford, William Cushley, Dorothy O'Donnell, Cecilia Cannon and Patrick Ferry. Departments of Biochemistry and Surgery, University of Glasgow, Glasgow G12 8QQ, Scotland.

Tumour draining lymph nodes are frequently removed in breast cancer patients to provide an index of the ability of the tumour to spread and invade other tissues. An invaded lymph node indicates a poor prognosis. Tumour cells from invaded nodes can be recovered and used for diagnostic probing to determine which population among the heterogeneous cells from the main tumour has metastatic potential.

We have analysed samples of such nodes in comparison to primary tumours from the same patient with respect to (i) growth factor requirements (ii) primary tumour cell heterogeneity (iii) detailed electron microscopic analysis of individual cells. Invasive cells are clearly shown to be a minor subpopulation of the main tumour.

D 405 CHEMOTACTIC FACTORS FROM EXTRACELLULAR MATRIX PREPARATIONS REGULATE ORGAN SPECIFIC METASTASIS. Robert F. Cerra, S. David Nathanson, Department of Surgery, Henry Ford Hospital, Detroit, MI 48202.

A selective pattern of metastasis, not accountable for by a simple mechanical trapping mechanism, is exhibited by many primary tumors and is regulated by properties of both the tumor cell and the host organ. This organotropism may be controlled, in part, by migration of an invading tumor cell toward organ specific chemotactic factors released from the connective tissue matrix as a result of proteolytic digestion. To understand the role chemotaxis plays in organ specific metastasis, we have examined 4M guanidine extracts of extracellular matrix (biomatrix) prepared from various organs. A quantitative measure of specificity (motility index) was determined, with an index greater than 1.00 indicating specificity. The murine liver colonizing cell line, B16-L4b, demonstrated only liver specific motility, while the lung colonizing B16-F10 and B16-BL6 lines showed only lung specific activity. No specific activity toward the nonspecific kidney extract was observed.

MOTILITY INDEX	KIDNEY	LIVER	LUNG
B16-L4b	0.57 ± 0.05	2.24 ± 0.20*	0.91 ± 0.2
B16-F10	0.39 ± 0.02	0.32 ± 0.19	2.76 ± 0.87*
B16-BL6	0.77 ± 0.22	0.19 ± 0.05	2.71 ± 0.46*

Gel filtration studies revealed the liver chemotactic activity elutes as four major peaks at Mr>2.5x10^6, Mr ~245,000, Mr ~ 120,000 and Mr ~37,000. The lung activity elutes as Mr~ 180,000 and Mr~90,000 molecular weight proteins. These results demonstrate organ specific chemotactic factors are present in the extracellular matrix of liver and lung and may serve to direct organ specific metastasis.

D 406 EFFECTS OF A RECOMBINANT TISSUE INHIBITOR OF METALLOPROTEINASES ON COLLAGEN DEGRADATION BY TUMOR CELLS, Ofelia A. Alvarez, David F. Carmichael and Yves A. DeClerck Division of Hematology/Oncology, Department of Pediatrics, Children's Hospital of Los Angeles and the University of Southern California 90027

Metalloproteinases with degradative activity for type I collagen, type IV collagen and gelatin, were identified in 3 tumor cell lines with different metastatic potentials. In all 3 lines, most of the proteolytic activity was recovered in a secreted form in the conditioned medium, whereas only minimal activity was found in the cytosol and cytoplasmic membranes. A c-Ha-ras transfected rat cell line, which had the highest metastatic potential in nude mice, had also the highest level of metalloproteinase activities and secreted several gelatinolytic proteases with molecular weights ranging from 200,000 to 50,000 Daltons. In contrast, a human melanoma cell line which did not metastasize in nude mice secreted mainly a gelatinase with a molecular weight of 67,000 Daltons. A recombinant inhibitor of metalloproteinases from human fibroblasts (rTIMP) was found to be a potent inhibitor of all these metalloproteinases and also to inhibit the degradation of collagenous substrates by the c-Ha-ras transfected cells, but not by the human melanoma cell line. Our data suggest that this inhibitor may be a potent inhibitor of invasion and metastasis of tumor cells that produce high levels of metalloproteinases.

D 407 **PHENOTYPICAL CHANGES DUE TO ACQUIRED CHEMORESISTANCE IN VITRO** Manfred Dietel, Hartmut Arps and Axel Niendorf, Institute of Pathology, Eppendorf University Hospital, Martinistr. 52, D 2000 Hamburg 20, Federal Republic of Germany

Acquired resistance to cytostatic drugs is one limitation of chemotherapy. This morphological study investigates phenotypic and cytogenitic differences of sensitive cells vs. the resistant variant derived therefrom.

Long-term culture of the gastric carcinoma cell line EPG85-257 and the adrenal carcinoma cell line EPA86-043 in stepwise increased concentrations of mitoxantrone (DHAD) and cisplatin (cPl) resulted in an enhanced IC_{50} of DHAD by 180 fold (EPG85-257) and of cPl by 130 fold (EPA86-043). Meanwhile the cells were supervised by interference and electrone microscopy, immunocytochemistry, kariotype analysis and DNA break determination.

Differences between sensitive and resistant EPG85-257 variants were found to be the creation of DHAD (shown by the blue staining) containing, excentric shaped surface vesicles with double membranes, appearance of the *mdr* glycoprotein P170 and of altered tubulin filaments with pearl-like condensations. The resistant EPA86-043 cells exhibited changes of the kariotype with several new marker chromosome and reduced DNA strand breaks.

In vitro acquired chemoresistance to cytostatic drugs was associated with definitive morphological alterations of the pheno- and kariotype. Whether these changes occur only in the cell lines investigated or represent a general mechanism remains to be clarified.

D 409 myc/ras COOPERATION IS INSUFFICIENT FOR METASTATIC TRANSFORMATION, Sean E. Egan, Maureen A. Spearman, James J. Broere, Alice B. Levy, Jim A. Wright and Arnold H. Greenberg, Manitoba Institute of Cell Biology, Winnipeg, Manitoba, R3E 0V9. We have previously reported that 10T½ cells transformed by ras in fetal calf serum are metastatic. In order to study the cooperative interaction of myc and ras in regulating the metastatic phenotype, we have transformed 10T½ through the sequential addition of myc and ras in dialyzed calf serum, a condition in which ras alone does not transform (Hsiao et al., MCB. 7:3380-3385, 1987). Surprisingly, the myc/ras cell lines were tumorigenic but completely non-metastatic. When myc and ras were sequentially introduced in fetal calf serum, approximately 25% of the cell lines were tumorigenic and metastatic. The lack of malignant potential in the majority of the myc/ras lines was not a result of myc suppression, because introduction of myc into a ras-transformed line did not inhibit metastasis formation. An additional event which can be promoted by a factor present in fetal calf serum is required for metastatic transformation. However, myc allows for benign transformation in cooperation with ras in the absence of the fetal calf serum promoted signal. In conclusion, myc/ras cooperation is sufficient for transformation but insufficient for induction of the full metastatic phenotype. This work is supported by the National Cancer Institute of Canada.

D 410 TUMOR PROMOTING ACTIVITY OF EPSTEIN-BARR VIRUS INDUCING FACTOR (EIF)/TRANSFORMING GROWTH FACTOR TYPE ß (TGF-ß), Michaela Götschl, P. Höfler and G. Bauer, Abteilung Virologie, Universität Freiburg,Hermann-Herder Str. 11, 7800 Freiburg, W-Germany.

Epstein-Barr Virus inducing factor (EIF) from platelets was first characterized by its ability to induce synthesis of Epstein-Barr virus antigens in latently infected lymphoid cell lines and to cooperate with chemical inducers of EBV antigen synthesis (1,2,3). EIF shows PDGF-like growth factor activity for certain fibroblast cell lines (4) and has transforming growth factor type-ß activity. One of its four active components is identical to TGF-ß1. The most interesting activity of EIF/TGF-ß is its involvement in multistep carcinogenesis in-vitro and in-vivo (5,6). EIF/TGF-ß acts as a potent complete tumor promoter in-vitro and exhibits convertogenic activity in-vivo. Initiation of C3H 10 T 1/2 mouse fibroblasts with UV light and tumor promotion with EIF/TGF-ß leads to the appearance of foci of stably transformed cells. The effect of EIF/TGF-ß is dose dependent. When higher doses of EIF are applied, the initiating agent is no longer required for the full carcinogenic effect. Cells stably transformed after promotion by EIF/ TGF-ß show the ability to grow in semisolid medium and are tumorigenic in athymic mice. The transformed state is maintained via an autocrinous loop, i.e. the cells secrete transforming growth factors and have acquired the ability to respond to them. The maintenance of transformation via autocrinous stimulation can be explained either by an epigenetic or a genetic model. DNA transfection experiments favour the genetic model. The phenotype of cells transformed by UV-light and EIF can be transferred to normal cells by transfection with genomic DNA. The recipient cells transiently acquire the ability to grow in semisolid medium. The possible roles of EIF/TGF-ß in the establishment and maintenance of transformation will be discussed.

1. Virology 121,184-194, 1982
2. J.Biol.Chem.257, 11405-11410, 1982
3. J. Biol. Chem. 257, 11411-11415, 1982
4. EMBO Journal 4, 1957-1961, 1985
5. J. Cancer Res. Clin. Oncology 109 (2), A 49, 1985
6. Fürstenberger et al. submitted

D 411 PREFERENTIAL SURVIVAL OF UNIQUE TUMOR SUBPOPULATIONS DURING LUNG IMPLANTATION AND INVASION WITH ALTERED MATRIX AND PROTEASE GENE EXPRESSION, M.C. Gingras, J.A. Wright and A.H. Greenberg, Manitoba Institute of Cell Biology, Winnipeg, Manitoba, R3E 0V9. To elucidate the selection processes that are associated with in vivo metastatic progression, 10T½ fibroblasts transformed by a plasmid containing H-ras and neo^R genes were injected intravenously into C3H/HeN mice, and recovered from lungs by enzymatic treatment and selective outgrowth in G418. Less than 1 in 10^3 cells survived in the lung 30 min. after inoculation and these exhibited a unique phenotype. This was characterized by a propensity to lodge in the lung on reinjection, markedly enhanced mRNA levels of procollagen α_2(I), procollagen α_1(III) and fibronectin, and decreased levels of laminin, MEP (procathepsin L), transin and H-ras. Between 1 and 9 days after tumor injection, the phenotype of the cells surviving in the lung changed dramatically and exhibited a pattern of gene expression similar to the injected tumor, but were now 26-fold more metastatic. Both the phenotype characterized by its propensity to arrest in the lung, and that showing enhanced metastatic ability were unstable on prolonged in vitro culture. We hypothesize that two selection events have occurred. The first is for lung arrest and implantation of variants of the injected tumor with high matrix protein and low protease levels. A second selection then occurs for tumor cells that carry a favorable phenotype for invasion and proliferation which is associated with low matrix protein and high protease gene expression. These two phenotypes are represented within a clonal population of recently transformed tumor cells. (Supported by the NCI of Canada)

D 412 CLONING OF THE HUMAN TYPE IV COLLAGENASE GENE AND MODULATION OF ITS EXPRESSION IN HUMAN FIBROSARCOMA CELLS (HT-1080) BY TGF-β, TPA AND EGF, Pirkko H. Huhtala[1], Jouko Lohi[2], Jorma Keski-Oja[2]and Karl Tryggvason[1],Biocenter and Department of Biochemistry, University of Oulu, SF-90570 Oulu, Finland and Department of Virology, University of Helsinki, SF-00290 Helsinki, Finland.

In our previous studies we have shown that type IV collagenase that specifically degrades basement membrane (type IV) collagen is closely linked with the metastatic phenotype. The enzyme is highly homologous with interstitial collagenase and stromelysin. However, in contrast to the latter, type IV collagenase has three copies of internal repeats that share high homology with the collagen binding domains of fibronectin. In order to explore further the regulatory mechanisms of basement membrane specific proteolysis in tumor invasion we have isolated the gene for human type IV collagenase. A 15 kb genomic clone was isolated and partially characterized. One exon of 149 bp corresponds exactly to exon 3 in the rat transin (stromelysin) gene and exon 3 in the human interstitial collagenase gene, indicating that the gene structure for mammalian proteinases is very similar. Preliminary studies on the structure of the region coding for the internal repeats of type IV collagenase indicate that the intron-exon boundaries differ from the correspondig boundaries in the fibronectin gene. Modulation of type IV collagenase gene expression was studied in HT-1080 cells and compared with that of u-PA another proteinase frequently increased in neoplasia. TGF-β caused a protein synthesis dependent increase in type IV collagenase mRNA whereas the changes in u-PA mRNA did not appear to be protein synthesis dependent. TPA and EGF had no effect on type IV collagenase mRNA levels. These studies indicate a different regulation pattern for these two proteinases that are important for tumor invasion.

D 413 GENETIC IDENTIFICATION OF HUMAN MELANOMA ASSOCIATED ANTIGENS WITH CASCADE ENRICHED PROBES, J.T. Hutchins, R.J. Deans, J. Kan-Mitchell and M.S. Mitchell, Depts. of Microbiology and Medicine, USC Cancer Center, Los Angeles, CA 90033.

The focus of immunological studies in human melanoma has been to develop an effective diagnosis and treatment for disseminated disease. In melanoma patients treated with a melanoma cell lysate together with a novel immunological adjuvant DETOX ™, we demonstrated increases in cell mediated and humoral immunity to melanoma associated antigens (MAA), with clinical regressions in approximately 20-25% of the patients. To genetically identify and characterize these MAA's we have generated lambda gt 11 cDNA libraries from 2 human melanoma cell lines (M1, M21). One hundred sixty-three positive melanoma cDNA clones were selected by plaque hybridization screening using a cascade enriched melanoma cDNA probe. This probe was generated by successive subtractive hybridization with excess mRNA derived from a lung carcinoma cell line (Lu-1) lacking MAA's (by cold target inhibition and cell surface staining studies), followed by single strand cDNA isolation on hydroxylapatite. Northern blot analysis using clone M3.50.1 identified a 6 kb mRNA molecule in melanoma cell lines (M3,M4) and transformed fibroblasts from a melanoma patient. Interestingly, this mRNA was also expressed by glioblastoma (U138MG) and breast carcinoma (8734) cells but not in normal spleen tissue, hematopoietic tumor cells (K562, Daudi) or the Lu-1 cell line. Preliminary sequence analysis showed no obvious homology with other known gene or protein sequences. We will further characterize the function and expression of the M3.50.1 clone, as well as other positive clones identified using this strategy. These studies represent a novel approach to characterizing MAA's and may be useful in the development of a recombinant vaccine for treating melanoma patients.

D 414 PROLIFERATION CONTROL IN HUMAN TUMOR CELLS: ELEVATED CASEIN KINASE II, MODIFICATION AND PROCESSING OF NUCLEOLIN (C23).

Olaf-Georg Issinger, Helge R. Schneider, Gerhard Fritz and Gerhard Seitz*, Institut für Humangenetik and Institut für Pathologie*, Universität des Saarlandes, D-6650 Homburg-3, FRG.

Casein kinase II (CKII), which initiates the whole process of rDNA transcription by phosphorylation of nucleolin, has been shown earlier to be elevated in rapidly growing cell cultures. Here we show that CKII activity is also enhanced in solid tumors, e.g. mamma carcinomas when compared to mamma parenchym from the same patients. The specific activities measured were 86 U/mg protein up to 510 U/mg protein in the carcinomas and 0.5 U/mg protein up to 64 U/mg protein in the corresponding non neoplastic tissue (i.e. mamma parenchym). Higher levels of CKII were also shown by (i) immunoblots and (ii) by immunofluorescense studies using a CKII- specific polyclonal antibody. Nucleolin has been shown to be a physiological substrate for CKII. By immunofluorescense studies both proteins have been localized in the nucleolus. During the course of rDNA transcription nucleolin is degraded by a specific protease into several lower mol. mass polypeptides among them a 60 kDa protein. Experiments with the tumor promoter okadaic acid indicate that right after the cleavage step the 60 kDa protein becomes dephosphorylated by either phosphatase 1 or 2A.

D 415 **PROGNOSTIC VALUE OF LYMPHOCYTE HOMING RECEPTOR AND S PHASE FRACTION IN NON-HODGKIN'S LYMPHOMA**, Sirpa Jalkanen, Heikki Joensuu and Pekka Klemi, Departments of Medical Microbiology, Radiotherapy and Pathology, University of Turku and Turku University Central Hospital, SF-20520 Turku, Finland

Lymphocyte homing receptors (HRs) mediate lymphocyte binding to high endothelial venules, and control their circulation between the blood and the lymphoid organs. The role of HRs and nuclear DNA content in spread and prognosis of non-Hodgkin's lymphoma was studied from paraffin-embedded tumor sections of 104 patients followed up for the minimum of 5 years after the diagnosis. HR expression was analyzed by staining with a monoclonal antibody Hermes-3, and DNA content by flow cytometry. Ten (10%) lymphomas were HR negative (HR-), 14 (13%) weakly (HR+/-) and 80 (77%) strongly positive (HR+). HR- lymphomas disseminated less often than HR+/- or HR+ lymphomas (p=0.03), and their prognosis was more favorable (p=0.03), although they often had a large S phase fraction (SPF) indicating a rapid proliferation rate. A large SPF (>12%) was strongly associated with an unfavorable histologic type in Working Formulation classification (p<0.0001) and poor survival (p=0.006), whereas DNA aneuploidy was not. The 5-year survival rate corrected for intercurrent deaths was 61% in lymphomas with SPF <12% or with HR-, but only 15% if SPF was >12% and HR+ (p<0.0001). In multivariate analysis stage (p<0.001), SPF (p=0.002) and HR (p=0.003) were the only independent prognostic factors. Lymphocyte HR and SPF analyses were superior to histologic classification in predicting outcome and biologic behaviour of the disease.

D 416 CSF-1 (M-CSF), A MACROPHAGE ACTIVATING CYTOKINE, AND ITS RECEPTOR MAY BE RESPONSIBLE FOR THE INVASIVE PHENOTYPES OF ADENOCARCINOMAS, IMPLANTING PLACENTA, AND ACTIVATED MACROPHAGES IN VIVO AND IN VITRO.
Kacinski BM[1,2,4], Foellmer B[3], Stanley ER[2], Carter D[4], Yee LD[1,2], Bloodgood RS[1], Oemar B[3], Gerald W[4,6], Jones M[1], Schwartz PE[3], Chambers JT[1], Chambers SK[2], Kohorn EI[1], Rohrschneider LR[5], and Rothwell V[6]. Depts. of Therapeutic Radiology[1], Obstetrics & Gynecology[2], Internal Medicine[3], and Pathology[4], Yale University School of Medicine, New Haven, CT; Department of Developmental Biology and Cancer[5], Albert Einstein College of Medicine, Bronx, NY; The Fred Hutchinson Cancer Research Center[6], Seattle, WA; and Department of Surgery[7], Oregon Health Sciences University, Portland, OR.

We have described elsewhere that fms-complementary transcripts (with or without co-expression of CSF-1 transcripts and proteins) are expressed in human (ovarian, uterus, breast, lung) adenocarcinomas in vivo and in vitro. In vivo levels of expression of these fms-complementary transcripts in ovarian and endometrial adenocarcinomas correlate very strongly with clinicopathologic features (high FIGO stage, grade) predictive of poor outcome and markedly elevated plasma CSF-1 levels were observed in ovarian carcinoma patients with active disease. Extension of these initial clinical observations to in vitro studies on subcloned ovarian and endometrial adenocarcinoma cell lines, revealed that CSF-1 stimulated 1) cell proliferation, 2) ^{3}H-dT thymidine uptake, 3) fos mRNA synthesis, 4) neutral protease production, and 5) invasion of an amnionic membrane basement membrane support at physiologically relevant concentrations (~1 nM). Agonist and antagonistic actions were observed for other cytokines (IL-3, GM-CSF, G-IFN) involved in macrophage activation. Additional histopathological studies on human tissue specimens revealed that the actively-invasive portions of the adenocarcinomas and the syncytiotrophoblast of implantation sites express many antigens (in addition to fms) commonly observed in activated macrophages, some of which could contribute to a "tissue invasive" phenotype.

Such observations have led us to propose a model in which epithelial tumor cell expression of the CSF-1 receptor (activated by cytokine produced by either stromal mesenchymal cells or tumor cells themselves) confers a set of phenotypic changes reminiscent of macrophage activation. These changes include cell proliferation, the ability to invade heterologous tissue structures, and responsiveness to other cytokines which modulate the "activated" phenotype of macrophages. This model also suggests that there may be a common molecular physiology of "invasiveness" in invasive cells of immune (i.e., macrophages) and non-immune (adenocarcinomas, syncytiotrophoblast) origin.

This work has been supported by ACS (CD-262), NIH (CA-47292, CA-09259), Swebilius Foundation, and Bristol-Myers research grants to BMK. Genetics Institute has generously provided recombinant human cytokines and valuable advice and assistance in their use.

D 417 **LOCALISATION OF PLASMINOGEN ACTIVATORS AND PLASMINOGEN ACTIVATOR INHIBITOR (PAI-1) IN LEWIS LUNG CARCINOMA, NORMAL MOUSE AND RAT TISSUES**, Peter Kristensen(1), Jens Eriksen(1), Leif R.Lund(1), Peter A.Andreasen(2), Charles Pyke(3) and Keld Danø(1). Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark(1), Dept.Biochemistry C, University of Copenhagen, Denmark(2), and Dakopatts, Glostrup, Denmark(3). The invasively growing Lewis Lung Carcinoma was analyzed using immunohistochemical and biochemical methods. Tumor cells in both primary tumors and metastases were found to contain urokinase-type plasminogen activator (u-PA) and plasminogen activator inhibitor (type 1) (PAI-1), but only very few cells contained tissue-type plasminogen activator (t-PA). The distribution of u-PA and PAI-1 in the primary tumors was generally similar. However, in all tumors analyzed, one or several areas showing a strong u-PA, but low PAI-1 immunoreactivity was found. The tumor cells in these areas were degrading the surrounding muscle tissue, these findings suggesting that the secretion of PAI-1 by the tumor cells may be playing a role in the regulation of the extracellular proteolysis in this tumor. In addition to the histological localisations previously reported in normal mouse and rat tissues, u-PA has now been found in epithelial cells situated luminally in the rat prostate epithelium and upon castration the number of these cells were found to increase strongly and correlated with an increase in u-PA mRNA. t-PA was found in somatostatin cells of islets of Langerhans and hypothalamus, and PAI-1 was found in thrombocytes, megakaryocytes and in the pituitary and adrenal medulla. In situ hybridization using an anti-sense cRNA probe has shown that the epithelium of the kidney distal and collecting tubules and of the bladder and a fibroblast-like cell in mouse fundus ventriculi, previously reported to contain u-PA immunoreactivity, synthesizes u-PA.

D 418 **MUC18, A MEMBER OF THE Ig SUPERFAMILY IS CORRELATED WITH THE FORMATION OF METASTATIC MELANOMA** *Jürgen M.Lehmann, Christine Sers, Gert Riethmüller and Judith P.Johnson, Inst.for Immunology, University of Munich, D-8000 Munich 2, F.R.of Germany*

The progression of a primary malignancy into metastatic disease is a complex multistep process. For a better understanding of this process, knowledge of the biochemical nature of the properties acquired by tumor cells during tumor progression should be of great value. MUC18 is a 113,000 dalton surface glycoprotein, whose expression is correlated with tumor progression in human malignant melanoma and which has yet not been identified in other tumors. In normal tissues its expression is limited to smooth muscle cells. cDNA cloning revealed that the MUC18 glycoprotein consists of 5 domains, all homologous to each other and to immunoglobulin-like molecules. Among the immunoglobulin related molecules the carcino embryonic antigen CEA (20% identity over 297aa), the myelin associated glycoprotein MAG (22% identity over 362aa) and the neuronal adhesion molecule N-CAM (26% identity over 137aa) showed greatest similarity. The 3 dimensional structure created by a folded sandwich of anti-parallel beta sheets stabilized by a disulfide bond and the repeat of this domain structure is thought to be essential for function as a receptor for soluble or cell bound factors controlling movement or differentiation. The association with melanoma progression suggests that the property reflected by MUC18 expression plays a role in the successful formation of metastatic disease.

D 419 **L-PLASTIN: A HUMAN LEUKOCYTE PROTEIN INAPPROPRIATELY EXPRESSED IN FIBROSARCOMAS AND OTHER SOLID TUMORS**

Ching-Shwun Lin[1], Ruedi Aebersold[2], Stephen Kent[3], Karabi Ghosh[1] and John Leavitt[1], Institute for Medical Research, 2260 Clove Drive, San Jose, CA 95128[1], Biomedical Research Center, University of British Columbia, Vancouver, 16T-W5[2], and Division of Biology, California Inst. of Technology, Pasadena, CA 91125[3]. The phosphoprotein, *L-plastin*, was originally identified as a transformation-induced protein of neoplastic human fibroblasts. L-Plastin was also found in many other tumor cell lines derived from human sarcomas and carcinomas, but it is not expressed in normal fibroblasts or cells of other solid tissues. L-plastin is normally expressed in leukocytes as one of the most abundant proteins; therefore its expression may play a role in hematopoietic cell differentiation. Cloning and characterization of two cDNAs encoding plastin has revealed the existence of two distinct isoforms of plastin, the "L" isoform mentioned above and the "T" isoform which is normally expressed in cells of solid tissues, but not in hematopoietic cells. Thus, this gene family is differentially regulated in neoplastic transformation like other gene families that encode abundant archetectural proteins such as the tropomyosins and actins. The induction of L-plastin is distinguished from these other gene modulations in that L-plastin represents a qualitative marker for human cancer cells with the exception that it was found in only about 50% of all tumor cell lines examined thus far. We have isolated genomic clones for both the L and T isoforms in order to study the mechanism of L-plastin gene activation in neoplasia. Preliminary findings suggest that gene rearrangement is not involved in this activation process. The L-plastin cDNA was inserted into the *beta*-actin promoter expression vector to allow expression of L-plastin in normal fibroblasts. Transfection of this plastin gene into rat fibroblasts led to a radical morphological change in the transfectant cells. We will present new hypotheses relating to the mechanism of activation of the human L-plastin gene in certain neoplasias and the relationship of neoplasia in solid tissues to hematopoietic cell differentiation.

D 420 IMMUNOHISTOCHEMICAL DETECTION OF CATHEPSIN B IN CARCINOMA IN SITU AND INVASIVE TRANSITIONAL CELL CARCINOMA OF THE HUMAN BLADDER. Brian C.-S. Liu and Jon A. Jacobson. Dept. of Urology and the Brookdale Center For Molecular Biology, Mount Sinai School of Medicine, New York, NY 10029.

During the metastatic cascade a tumor cell passes through several connective tissue barriers. These connective tissue barriers consist primarily of proteins such as collagen, glycoproteins, and proteoglycans. The expression and/or secretion of proteolytic enzymes by the tumor cells should, therefore, facilitate tumor invasion. Lysosomal cathepsin B can degrade components of the extracellular matrix. Using paraffin embedded tissue specimens and avidin-biotin immunoperoxidase technique, we now report the expression of cathepsin B in the tumor cells of invasive human bladder cancer but not in cells of carcinoma-in-situ.

Paraffin embedded tissues from patients with both areas of invasive tumor and areas of carcinoma-in-situ were used and were deparaffinized. Polyclonal sheep antibodies against human cathepsin B (Miles Scientific) were applied to the slides and followed with biotinylated rabbit anti-sheep immunoglobulin (Vector Labs). Avidin-biotinylated peroxidase and substrate solution were used to visualize the area of reactivity.

Our results indicate that in the invasive regions of each patient's biopsy materials, we have detected the expression of cathepsin B. However, in the patient's area with carcinoma-in-situ, there was non-detectable expression of cathepsin B. This suggests that there is a biological difference between invasive and in-situ carcinoma in their capability to degrade basement membrane and therefore their ability to invade and metastasize, and may further suggest a difference in the developmental pathways for the different forms of human bladder cancer.

D 421 TRANSACTIVATION OF POL II AND III PROMOTERS BY SV40 SMALL t ANTIGEN.

Mary R. Loeken (1,2), Ilan Bikel (2,3), David M. Livingston (2,3), and John Brady (4). 1 Joslin Diabetes Center; 2 Harvard Medical School, Boston, MA; 3 Dana Farber Cancer Institute; 4 Laboratory of Molecular Virology, NIH, Bethesda, MD.

Small t antigen (t), an early gene product of SV40, is not strictly required for either viral lysis or transformation in cultured cells. However, t facilitates transformation brought about by limiting cellular levels of large T antigen (T). An explanation for this activity and for conservation of t structures among papovaviruses has not been evident. Previously we demonstrated that a plasmid expressing the SV40 T and t antigens could transactivate the Ad E2 promoter (Loeken, et al., Mol. Cell Biol., 1986). Here we report that plasmids which express t, in the absence of T, can transactivate the enhancer of the Ad E2A promoter. Other Pol II promoters, such as the SV40 late promoter and the HTLV I LTR were not transactivated by t alone. Co-transfection of E2CAT DNA with plasmids expressing t resulted in increased steady state CAT RNA levels. SV40 large T also transactivated pE2A-CAT. Both t and T were each able to markedly transactivate the Pol III promoter of the Ad VA I gene. These common effects could be due, at least in part, to a structure within the common 82 a.a. sequence shared by T and t. However, the common sequence is not sufficient on its own, since deletion of all of the t unique sequence, or only the 111-174 a.a. sequence abolished activity. A role in viral infection for t may be to facilitate the action of T on the SV40 late promoter, especially when T levels are low. Thus, the helper role of t in transforming infection and during viral persistence in its natural host may be, in part, due to its transactivation function operating when that of T is inefficiently expressed.

D 422 ISOLATION AND CHARACTERIZATION OF A NEW GENE FOR A Ca^{++}-BINDING PROTEINS WHICH IS SPECIFICALLY EXPRESSED IN DIFFERENT METASTATIC CELLS, E.Lukanidin, A.Ebralidze, E.Tulchinsky[1], M.Grigorian, V.Senin[2], E.Revasova[2], Institute of Molecular Biology, [1]Institute of Developmental Biology, [2]All Union Cancer Research Center, Moscow, USSR

The method of molecular cloning coupled to differential DNA reassociation was used for isolation of the gene (mts1) which is specifically expressed in metastatic cells. The level of expression of gene mts1 is correlated with metastatic potential of tumor cells. The cells which have high level of mts1 gene expression demonstrates high extent of metastasis. The data suggests a role of mts1 gene in regulation of metastatic behaviour of tumor cells. Transcription of mts1 gene was found not only in tumor, but in normal cells. Homologous RNA was found only in spleen, thymus, bone marrow and blood lymphocytes. DNA sequencing of mts1 gene revealed open-reading frame containing the information for peptide of 101 amino acids. Comparison of amino acid sequences of the mts1 gene product revealed 55% homology with S100 Ca^{++}-binding protein. Especially high homology was found in loop part (residues 21-33 and 63-74) of the domain (66%). Thus, the mts1 protein is a new member of calcium modulated protein family.

D 423 THE BIOLOGICAL POTENTIAL OF EARLY PROLIFERATIVE HEPATIC LESIONS INDUCED BY A PEROXISOME PROLIFERATOR. Daniel S. Marsman and James A. Popp, Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709. Several laboratories have established the potent hepatocarcinogenicity of Wy-14,643 (WY), a hypolipidemic peroxisome proliferator. Previous work from this laboratory has reported that WY-induced basophilic foci/nodules exhibit numerous mitotic figures, rapid growth in size and compression of surrounding tissue. Because these findings were suggestive of early hepatocyte transformation, the current study was designed to determine the biological fate of early proliferative lesions induced by WY. Male F344 rats (20/group) were fed WY (0.1%) or basal diet for 22 or 37 weeks, and additional WY-fed rats were subsequently returned to the basal diet for up to 52 weeks. As expected, liver weights in WY-fed rats were ~50% higher than controls at 22 and 37 weeks, with liver weights returning to control values after 15 weeks of basal diet. No significant gross or H&E microscopic lesions were observed at 22, 37 or 52 weeks among controls, nor were any lesions observed after 22 weeks of WY-diet. In contrast, 37 weeks on WY-diet resulted in a 25% incidence of rats with hepatic nodules >12mm in diameter. Numerous basophilic foci/nodules were also observed microscopically with a volume density accounting for 0.5% of the hepatic parenchyma, having a mean focal volume of 1.5mm and a numerical density of 7.0 lesions/cm^3. However, 15 additional weeks of feeding the basal diet caused complete regression, with no significant gross or microscopic lesions identified in this WY-fed group. These results demonstrate that early-stage lesions induced by the peroxisome proliferator and potent carcinogen WY, although appearing morphologically well advanced, undergo a rapid reversion similar to the surrounding liver. Further analysis of additional time points is necessary to define the biological potential of altered hepatocytes to allow direct comparison to autonomous tumors induced by other types of carcinogens.

D 424 SPONTANEOUS TRANSFORMATION OF MOUSE CELLS BY IN VIVO TRANSFER OF DNA FROM A HUMAN TUMOR XENOGRAFT.
Vivi Flørenes, Ola Myklebost, Alexander Pihl and Øystein Fodstad, Departments of Tumor Biology and Biochemistry, Institute of Cancer Research, Montebello, 0310 OSLO 3, Norway, tel (-47-2-) 506050

During a study of human non-small cell lung cancers transplanted into nude mice, a late secondary tumor arose in a mouse cured of the original tumor. The secondary tumor was histologically similar to the human tumor and was first thought to be a spontaneous metastasis. However, immunological and cytogenetic examination showed it to be of murine origin. The murine tumor cells were shown to contain human DNA fragments detectable with human-specific repeat sequences as probe. Such spontaneous transfer of genetic material from human tumor cells in vivo has, to our knowledge, not been described previously. It seems likely that the human DNA has transferred to the murine cells genetic information coding for the growth pattern and phenotype of the human tumor. We have now cloned the human sequences from the mouse tumor cells in attempts to identify the genes involved and elucidate the mechanism by which the human DNA was transferred.

D 425 THE EFFECTS OF HOST INFLAMMATORY CELLS ON TUMOR PROGRESSION,
Futoshi Okada, Jun-ichi Hamada, Junji Hasegawa, Noritoshi Takeichi, Masuo Hosokawa and Hiroshi Kobayashi, Laboratory of Pathology, Cancer Institute, Hokkaido University School of Medicine, Sapporo, Hokkaido 060
We investigated the effects of host inflammatory cells on the progression of QR regressor tumor cells in C57BL/6 mice. The inflammatory cells were produced by the implantation of sterilized hemostatic spongel into the peritoneal cavity of mice. We noted an enhanced tumorigenicity of QR cells ($1x10^5$ cells) after they had combined with the inflammatory cells ($1x10^6$ cells) when 3 out of 8 mice developed tumors. We have already noted a close correlation between the in vivo tumorigenicity and the in vitro PGE_2-production of QR regressor tumor cells. We were able to estimate, therefore, the progression of QR cells by an examination of PGE_2-production after co-culturing the QR cells with the inflammatory cells: we found high levels of PGE_2-production (16000 pg/ml) by QR regressor tumor cells after they were co-cultured with the inflammatory cells, while the observed PGE_2-production in QR regressor tumor cells ($1x10^4$ cells) alone was 1300 pg/ml and in inflammatory cells alone ($1x10^6$ cells) was 960 pg/ml. One of the mechanisms involved in the progression of QR regressor tumor cells by co-existence with inflammatory cells is thought to be the production of oxygen radicals from the inflammatory cells. We conclude that tumor progression may be enhanced by host inflammatory cells.

D 426 IMMUNOGLOBULIN HEAVY CHAIN GENE REARRANGEMENTS IN CLL: CORRELATION WITH CLINICAL STAGE.
Rechavi G,*Katzir N, Brok-Simoni F, Mandel M, Laks Y,*Gurfinkel N,*Givol D, Ben-Bassat I & Ramot B. Institute of Hematology, The Chaim Sheba Medical Center, Tel Hashomer and Sackler School of Medicine, Tel-Aviv University and Department of Chemical Immunology,*Weizmann Institute of Science, Rehovot, Israel.
A search for possible correlation between the clinical stage of chronic lymphocytic leuekmia (CLL) and the pattern of immunoglobulin heavy chain gene rearrangements was undertaken. DNA samples from the leukemic cells of 38 CLL patients were analysed by Southern blot hybridization. Using probes for the immunoglobulin heavy chain J (IgHCJ) and Cμ (IgHCμ)regions a marked heterogeneity of hybridization patterns was observed. The number of IgHCJ hybridization bands varied from one to four and more than two hybridizing bands were found in 58% of the patients. In 42% of the patients no germline IgHCJ genes were found. In 34% of the patients additional IgHCμ bands were observed varying from one to three. A Cμ germline was preserved in all samples. There was no correlation between the clinical stage and the number of hybridizing IgHCJ bands. However, a significant correlation was found between the loss of IgHCJ germline band or a Cμ multiband and progressive stage of the disease. These genetic events in the immunoglobulin genes of advanced CLL patients are assumed to result from clonal evolution and tumor progression.

D 427 "GENETICS OF SPONTANEOUS AND CHEMICALLY INDUCED MURINE HEPATOCARCINOGENESIS" L.V. Rodriguez[1], L.S. Ramagli[1], D.A Johnston[2], H.A Dunsford[3], F.F. Becker[1] and J.E. Womack[4]; [1]Depts. of Molecular Pathology and [2]Biomathematics, M.D. Anderson Cancer Center, Houston, TX., 77030; [3]Dept. of Pathology, Univ of Texas Medical Branch at Galveston, Galveston, TX. 77550; [4]Dept. of Veterinary Pathology, Texas A&M University, College Station, TX. 77843. Tumorigenesis analysis in inbred mice indicate genetic factors predispose to spontaneous and chemical hepatocarcinogenesis susceptibility . Our studies in a mouse model seek the genetic basis for spontaneous and induced carcinogenesis. The model's progenitor strains C3H/HeN and C57BL/6N vary drastically in spontaneous hepatoma incidence and susceptibility to chemical hepatocarcinogenesis. Analysis of genetic predisposition was limited since the population parameter tumor incidence cannot be evaluated in segregating F_2 and backcross generation individuals. We constructed 7 Recombinant Inbred Strains (RIs) from a segregating B6C3 F_2 generation to study carcinogenesis genetics. RIs were inbred to homozygosity and Strain Distribution Patterns (SDPs) for 20 biochemical loci on 12 chromosomes differing in C3H and C57 determined. Spontaneous hepatoma in RIs segregated into 3 phenotypic groups in a ratio of 4:2:1 for high-intermediate and low incidence, best fitting a 2-3 gene tumorigenesis model with genes expressing unequal effects. We also investigated genetics of diethylnitrosamine (DEN) susceptibility. Liver tumor multiplicity was used as a quantitative genetic trait and at 40 weeks post DEN exposure RIs segregated into 3 phenotypic groups indicating control by 2-3 genes. Chemical susceptibility SDPs differed considerably from spontaneous carcinogenesis SDPs. Therefore, different sets of genes control predisposition to spontaneous incidence and induced hepatoma susceptibility.

D 428 HUMAN OSTEOSARCOMA TRANSFORMED BY ras: NEW MODEL FOR STUDIES ON HEMATOGENOUS METASTASIS Dvorit Samid and Raya Mandler Department of Pathology, Uniformed Services University of the Health Sciences. Bethesda, MD 20814
Hematogenous metastasis to the lungs is a major complication in patients with different types of sarcomas. So far, studies have been hindered because of lack of appropriate human cellular model systems. We have now established subclones of a human osteosarcoma (HOS) transformed by v-Ki-ras oncogene, suitable for experimental metastasis studies in nude mice. Cells transplanted i.v. formed pulmonary metastases in 80%-100% of recipient mice with a median number of 20 nodules/animal. The metastatic foci could be easily detected as macroscopic nodules 30-40 days post inoculation. Detection of smaller tumors was improved by the use of selectable geneticin-resistant cells, obtained by transfection with pSV2neo DNA. The ability of the sarcoma cells to perform the later steps of blood-borne metastasis correlated with elevated biosynthesis and activity of urokinase-type plasminogen activator (uPA), a serine protease implicated in degradation of extracellular matrix and tumor invasion. The development of the human sarcoma model is essential and timely in view of the growing awareness that human tumor cell lines (and not animal tumors) of different histologic origin should be used to identify mechanisms of metastasis and therapeutic agents with histotype-specific activities.

D 429 ESTROGEN INCREASES THE NATURAL KILLER CELL-SUSCEPTIBILITY OF BREAST CANCER CELLS. Isabella Screpanti, Alberto Gulino, Elena Toniato, Angela Santoni and Luigi Frati, Department of Experimental Medicine, Univ. of Rome and Department of Sciences, Biotechnology and Biometrics, Univ. of L'Aquila, Italy.
Estrogens play a central role in mediating the development and progression of hormone-dependent breast cancer by increasing cell proliferation and the malignant phenotype resulting in the increased invasiveness of the tumor cells. Tumor invasiveness and dissemination has been shown to be controlled by natural killer cells (NK). Malignant phenotype associated with oncogene-induced transformation has been reported to be paralleled by increased NK-susceptibility. In this study we have investigated the effect of estrogen on NK susceptibility of MCF-7 breast cancer cells. Human peripheral mononuclear blood cells from healthy donors were isolated by Ficoll-Hypaque and nylon-non-adherent lymphocytes were cultured for 18 h with recombinant IL-2 (100 U/ml). At the end of the incubation the lymphocytes were tested for NK activity in a 18 h ^{51}Cr release assay against ^{51}Cr-labeled MCF-7 cells previously cultured with or without 50 nM estradiol (E2) for 1, 3 and 5 days. E2 treatment of MCF-7 cells induced an increase of their sensitivity to the lysis by NK cells. This increase was already observed after 24 h treatment and reached a maximum level (50-60%) after 3 to 5 days. Treatment of lymphocytes with a monoclonal antibody directed against CD16 antigen mainly expressed on NK cells and granulocytes completely abrogated the cytotoxic activity against MCF-7 cells. The estrogen receptor-negative breast cancer cell line BT-20 was insensitive to E2 effect. We conclude that estrogen can modulate the mechanisms of natural cytotoxicity against breast cancer at the level of target cells.

D 430 IDENTIFICATION OF GENES WITH DIFFERENTIAL LEVELS OF EXPRESSION IN MALIGNANT AND NON-MALIGNANT BREAST TUMOURS, Matthew G.F. Sharp, Susan M. Adams, Jennifer M. Varley and William J. Brammar, ICI Joint Laboratory, University of Leicester, Leicester LE1 7RH, U.K. Our aim was to isolate and identify cDNA clones which correspond to genes which are expressed at different levels in a malignant (carcinoma) and a non-malignant tumour of the breast (fibroadenoma). Carcinomas of the breast are characterised by rapid proliferative growth and frequent metastasis formation at distant sites, whereas fibroadenomas are entirely benign lesions which although proliferative, do not form metastases. It is the potential ability of breast carcinomas to metastasize, when compared to a benign lesion such as the fibroadenoma which makes the comparison between the two tumour types interesting. cDNA libraries derived from both tumour types were screened separately with probes obtained from the mRNA of carcinoma and fibroadenoma biopsies. Clones which appeared homologous to sequences expressed at different levels in the two tumour types were selected and scrutinized more rigorously. Individual patient variations were eliminated by subsequent multiple rounds of screening using paired fibroadenoma and carcinoma samples. This approach has been used successfully to isolate 18 carcinoma-derived clones and 13 fibroadenoma-derived clones which are associated with altered levels of gene expression in the two contrasting disease states. Of these, 20 were expressed at higher levels in carcinomas and 11 in fibroadenomas. The cDNA sequences of some clones have been found to correspond to known human sequences by computer-aided homology searches, whereas other clones remain unidentified.

D 431 DETECTION OF TPR-MET GENE REARRANGEMENT IN HUMAN CELL LINES BY TRANSCRIPT AMPLIFICATION, Neela R. Soman[1], Gerald N. Wogan[1] and J. S. Rhim[2], [1]Program in Toxicology, Whitaker College of Health Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139; [2]Department of Health and Human Services, National Institute of Health, Bethesda, MD 20894

We have developed a rapid and sensitive method based on polymerase chain reaction (PCR) for the detection of *tpr-met* oncogenic rearrangement in human cell lines. The *tpr-met* transforming sequence was isolated from an N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) treated, tumorigenic derivative (MNNG-HOS) of human osteosarcoma cell line (HOS). Activation of the *met* protooncogene occurred via a chromosomal DNA rearrangement in which the *met* locus on chromosome 7 was fused to the *tpr* locus on chromosome 1, resulting in the expression of a novel hybrid RNA.
Complementary DNA (cDNA) was generated from cellular transcripts using one of the PCR primers and was used as a template for PCR amplification of a 205 bp region carrying the breakpoint. An end-labeled internal probe (20-mer) was hybridized to an aliquot of the PCR reaction product. The amplified DNA was separated from excess primers on a native polyacrylamide gel, and detected by autoradiography. The 205 bp breakpoint region was detected in each of the three MNNG-transformed HOS cell lines tested, but not in the parental cell line or in 7,12-dimethylbenzanthracene or 3-methylcholanthrene transformed cell lines. This method is highly sensitive and can detect the *tpr-met* rearrangement using picogram quantities of mRNA as template.

D 432 **CLONING OF P3.58, A CELL SURFACE ANTIGEN CORRELATED WITH TUMOR PROGRESSION IN MELANOMA: IDENTITY WITH LYMPHOCYTE ADHESION MOLECULE ICAM-1.** **Barbara Stade, Bernhard Holzmann, Wilhelm Schwäble, Gert Reithmüller, Judith P. Johnson, Institute for Immunology, Univ. Munich, Munich 2 West Germany.**
Expression of the P3.58 antigen, gp89, by malignant melanoma correlates with tumor progression. While benign nevi and thin melanomas which have a good prognosis are rarely positive, approximately 70% of all tumors with a thickness greater than 1mm express P3.58. Clone λ89-1 was isolated from a melanoma cDNA library using monoclonal antibodies prepared against isolated denatured gp89. In Southern analyses with DNA from melanoma, mouse L cells and a P3.58+ L cell transfectant, λ89-1 hybridized to fragments which were identical in the melanoma and the P3.58+ transfectant. These fragments were not present in the L cell. Sequencing of λ89-1 revealed identity with the leukocyte intercellular adhesion molecule, ICAM-1. Northern and Southern analyses, as well as DNA sequencing, indicated no differences between melanoma cells and myeloid cells in the P3.58/ICAM-1 gene or its mRNA. The molecules precipitated from these cells differ in size presumably due to differences in N linked glycosylation but it is not yet known whether this results in functional variation. As it is a ligand of the lymphocyte function associated antigen 1 (LFA-1), P3.58/ICAM-1 may be involved in the development of metastases by increasing heterotypic adhesion between tumor cells and mobile infiltrating leukocytes. Furthermore P3.58/ICAM-1 contains the typical consensus sequence residues of the C3b/C4b binding protein family. The possible involvement in C3b binding suggests that P3.58/ICAM-1 may also help protect the tumor cells from immune destruction.

D 433 THE ROLE OF MAST CELLS IN TUMOR-ASSOCIATED ANGIOGENESIS, Jean R. Starkey and Patricia K. Crowle, Departments of Microbiology and Biology, Montana State University, Bozeman, MT 59717.
We have shown that the rate of development of tumor-associated angiogenesis is slower in mast-cell-deficient W/W^v mice compared to their +/+ mast-cell-sufficient littermates (Int. J. Cancer:42, 48-52, 1988). W/W^v mice also develop fewer spontaneous lung metastases, and bone marrow repair of the W/W^v mast cell deficiency restores both the tumor-associated angiogenic response and the incidence of spontaneous lung metastases. Mast cells were grown in culture from +/+ bone marrow and used to locally repair the ears of W/W^v mice. The tumor-associated angiogenic response was restored when a B16BL6 tumor was grown in the repaired ears and its intensity was roughly proportional to the number of mast cells present at autopsy. The rate and character of the vascular outgrowth obtained when rings of rat aorta were cultured within collagen I gels were used as an *in vitro* assay to dissect the role(s) of mast cells in tumor-associated angiogenesis. The presence of mast cells within the gel led to a more rapid cellular outgrowth while the presence of B16BL6 tumor cells or phorbol ester (PMA) led to the development of more mature vessel-like structures. Addition of heparin to the medium promoted single cell invasion of the collagen gels but did not promote maturation of the putative vessels. These studies indicate that mast cells are important to tumor angiogenesis and that this can be studied in culture.

D 434 AN ABNORMALLY EXPRESSED PROTEOGLYCAN CORE PROTEIN GENE IN CHRONIC MYELOGENOUS LEUKEMIA, Christine M. Stellrecht, Wendy M. Mars, Hisashi Gondo and Grady F. Saunders, Department of Biochemistry and Molecular Biology, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030.
Chronic myelogenous leukemia (CML) is a myeloproliferative disorder that is believed to result from a primary defect in a hematopoietic stem cell capable of multilineage differentiation. In an effort to understand the molecular events occurring in this disease, preferentially expressed genes in CML were previously selected and a cDNA clone, pD-D1, was obtained. To further elucidate the involvement of this gene in CML, its expression was analyzed in normal and leukemic samples and its nucleic acid sequence was determined. The results of the northern blots revealed that this gene was consistently expressed in CML total RNA samples. When compared with *in situ* hybridization analysis in the individual leukocytes, it indicated that this is most likely due to an over expression in the granulocytes which is the cell lineage that is primarily expanded in CML. The nucleic acid sequence was determined from a ~1.2 kb clone obtained from an acute myelogenous leukemia cDNA library probed with pD-D1. This sequence revealed a 100% homology at the 5' end to a proteoglycan (PG) core protein cDNA isolated from an HL60 cDNA library and contained an additional 533 bp 3' to this region. These results, implicating a consistent over expression of this PG core protein gene in CML, along with the recent reports of proteoglycans being involved in normal hematopoiesis and having the ability to absorb colony stimulating factors, suggest that this gene and gene product may be involved in the pathogenesis of this disease.

D 435 LOSS OF THE NORMAL c-Ha-*ras* ALLELE IN TWO MOUSE SKIN CARCINOMA CELL LINES CONTAINING AN ACTIVATED c-Ha-*ras* ONCOGENE, Richard D. Storer, Brian J. Ledwith, Karen R. Leander, Henry L. Allen, Andrew R. Kraynak, and Matthews O. Bradley, Merck Institute for Therapeutic Research, West Point, PA 19486. We have derived 2 cell lines from benign and 2 from malignant skin tumors induced by 10 nmol DMBA followed by 3.4 nmol of TPA twice weekly. Both papilloma lines contained a mutation in the 61st codon of the c-Ha-*ras* gene as diagnosed by XBA I restriction polymorphism. The two lines from carcinomas contained the same mutation. However, the 12 kb Xba I band with the normal allele of the c-Ha-*ras* gene was missing in both carcinoma cell lines. These data suggest that loss of the normal allele of the c-Ha-*ras* gene may be associated with malignant conversion in cells carrying a mutant *ras* allele. In nude mice, the papilloma lines produced benign cystic tumors, well- and poorly-differentiated basal cell carcinomas, and pure spindle-cell tumors resembling fibrosarcomas. In contrast, all tumors arising after injection of the carcinoma lines were spindle cell tumors. All malignant tumors arising s.c. in nude mice showed similar rearrangements in and amplification of c-Ha-*ras* sequences on southern blots. DNA fingerprinting analysis of the cell lines and nude mouse tumors revealed some evidence of genomic rearrangement and DNA amplification in tumor DNA but did not suggest fusion of injected cells with host cells as an explanation for the fibrosarcomatous appearance of many tumors. These data suggest that the malignant progression of initiated epidermal cells containing a single, activating point mutation may be associated with additional changes (conversion to a homozygous or hemizygous state, gene rearrangement, and gene amplification) involving the c-Ha-*ras* gene that correlate with tumor morphology.

D 436 STUDIES ON THE BIOLOGICAL ROLE OF AN EPITHELIAL DIFFERENTIATION ANTIGEN EXPRESSED BY SMALL CELL LUNG CANCER (SCLC) CELLS. E. Tagliabue, A. Ciavolella, S. Fasolato*, S. Martignone, S. Ménard, M.I. Colnaghi. Istituto Nazionale Tumori, Milano, *Università di Padova, Padova.

Different studies carried out on SCLC cell lines have allowed the identification of neural and epithelial-related antigens on the tumor cell surface. To investigate the possible functional role of the expression of these antigens, lung, lymphnode and bone marrow biopsies from 63 SCLC patients were tested by I.F. using different MAbs and then the obtained results were correlated with the disease progression. A life table analysis showed a statistically significant shorter survival time for SCLC patients with tumors expressing CaMBr1, an epithelial differentiation antigen recognized by the MBr1 MAb, than for patients with tumors which did not (log rank test: 2 =7.08, p 0.01. Different tumor agressivness or responsiveness to the therapy of the MBr1 positive patients could be responsible for this shorter survival time. To discriminate between the two hypotheses, the growth capacity in athymic mice of the tumor cells obtained from 25 SCLC biopsies were analyzed. The results indicated a highly statistically significant correlation (p 0.01) between the presence on tumor cells of CaMBr1 and the ability of these cells to grow in athymic mice. These data seem to indicate a CaMBr1 specific role in tumor agressiveness which we are now investigating.

D 437 CULTURED KAPOSI'S SARCOMA CELLS PRODUCE ACTIVATORS OF ENDOTHELIAL CELL INVASIVENESS Erik. W. Thompson[1], Thomas B. Shima[1], Adriana Albini[1], Shuji Nakamura[2], S. Zaki Salahuddin[2], Robert C. Gallo[2] and George R. Martin[1]. [1]LDBA, NIDR and [2]LTCB, NCI, NIH, Bethesda, MD 20892.

The chemoinvasion assay performed in a modified Boyden chamber measures the ability of the cells to migrate through a layer of reconstituted basement membrane gel (Matrigel). Long term cultured spindle cells (AIDS-KS cells) derived from human Kaposi's sarcoma lesions[1] were found to be highly invasive when compared to normal human endothelial, smooth muscle and fibroblastic cells. Furthermore, when embedded in a 3 - dimensional gel of Matrigel, these cells were highly invasive, typical of metastatic cells. Serum free conditioned medium from cultured Kaposi's sarcoma cells (KS-CM) stimulated basement membrane invasiveness of human umbilical vein endothelial cells.

Chemotaxis was measured in the same chamber, under the same conditions, without the Matrigel barrier. Chemotaxis of the normal vascular endothelial cells to AIDS-KS cell conditioned media was not stimulated by the KS-CM, compared to a fibroblast conditioned media control. This indicates that these effects specifically relate to the molecular machinery of invasiveness rather than overall mobility. Basement membrane invasion is mediated by type IV collagenase, and stimulated by laminin, a major basement membrane glycoprotein. AIDS-KS cell invasiveness, and endothelial cell invasiveness induced by Kaposi's sarcoma secreted products, could be inhibited by the active laminin pentapeptide YIGSR, and also by an inhibitor of collagenase type IV (SC44463; Searle). These data implicate basement membrane invasiveness in the pathophysiology of KS, supporting the theory that vascular and / or lymphatic endothelial cells are recruited into the lesional area by the KS cell products. Specific inhibitors of basement membrane invasion, such as YIGSR and SC44463, offer a possible strategy for the treatment of Kaposi's sarcoma. [1]Salahuddin et al, Science, in press.

D 438 MOTILITY IN RELATION TO INVASIVE CAPACITY,Nicolas A.F. van Larebeke, Walter De Coster, and Marc M. Mareel, Laboratory of Experimental Cancerology,Department of Radiotherapy, University of Gent,Belgium. Different pairs of related invasive and non-invasive cell lines were studied as to their motility in vitro on tissue culture plastic. It was not possible to describe a common characteristic that would distinguish cell lines with invasive capacity from non-invasive cell lines. Cell membrane ruffling was more pronounced in the invasive MDCK-T4 , NMD8/800 /10 T, MDCK F3 B9 and MDCK F3 B1, Rac -5 E, MCF-7/6 and transformed late passage MLE cells than in the parental or related MDCK-AZ dog kidney, NMuMG mouse mammary, MDCK F3 B5 cloned dog kidney, Rac -11P mouse mammary, MCF -7 human mammary tumor, or early passage mouse lens MLE cells. After manual delineation of cell contours on video-prints made at defined time intervals the translocation distance of the geometric center of gravity of a cell was measured using an automatic image analysis system. Stationary motility was measured using the corrected constant area parameter used by Bracke et al. (Clinical and Experimental Metastasis,in press), which shows a simple mathematical relation (corrected constant area= 100x (1-d shape))to the d-shape change factor described by Verschueren and van Larebeke, Cytometry,5,557 (1984). Different couples of related invasive and non invasive cell lines were studied. Rac-5E cells , the invasive ras-transfectant MDCK-T4 and transformed late passage MLE cells displayed both more translocation and faster shape change than related Rac -11 P, parental MDCK-AZ or parental early passage MLE cells. Invasive NMD8/800/10T cells showed , in the first 24 hours after plating, faster shape change than parental NMuMG cells. Invasive human HBL-100 mammary gland cells showed more translocation and faster shape change than non-invasive MCF-7 cells. Tangeritine, a flavonoid that inhibits invasion by MO4 mouse cells, appeared to slow down translocation and shape change. ET-18-OCH3, an alkyl-lysophospholipid derivative that induces invasion of HSU baby rat kidney cells in precultured embryonic chick heart fragments, induces, 24 hours after start of treatment, more translocation and faster shape change in these cells .

D 439 MONOCLONAL ANTIBODY CHARACTERIZATION OF CLONED REVERTANTS OF METASTASIS. Nissi M. Varki, Tien Vu and Leita Estes. Cancer Center, University of California, San Diego, La Jolla, CA 92093-0812.

The current understanding of the pathogenetic mechanisms of the metastatic disease process has been culled from a large quantity of clinical and pathological material, and also from extensive research, primarily utilizing transplantable rodent neoplasms. Most of these research endeavors have focused on the study of high metastatic variants. The occurrence of revertants, neoplastic cells that have lost the capacity to establish as metastases, has been observed in a few studies. In this report, we show the derivation and characterization revertants of metastasis in a human lung carcinoma model system. The athymic mouse derived metastatic variant MV522, of a human lung carcinoma cell line UCP3, was cloned in soft agar with eight resultant clonal subpopulations of cells. Monoclonal antibodies were used to analyze the cell surface properties of these cell lines. The capacity to spontaneously metastasize from subcutaneous sites in athymic mice was also assessed. Revertants of metastasis were thus isolated and characterized. A systematic analysis of the occurrence of reversion to a less malignant phenotype is now possible with these derived cell lines. When such mechanisms are fully understood, therapeutic modalities may then be designed that will encourage this process of reversion, thus aborting the usual inoxerable progression of a malignant neoplasm to metastases.