CELLULAR AND MOLECULAR BIOLOGY OF TUMORS AND POTENTIAL CLINICAL APPLICATIONS

John D. Minna and W. Michael Kuehl, Organizers

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Plenary Sessions

Poster Sessions

Late Additions

Oncogenes and Oncogene Activation

A0 ACTIVATION OF THE MYC ONCOGENE IN B AND T CELL NEOPLASIA, Suzanne Cory, The Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.

The c-myc gene has been strongly implicated in lymphoid neoplasia. The oncogenic potential of c-myc can be triggered in several different ways. In retrovirus-induced avian B lymphomas and a proportion of T lymphomas, proviral insertion near c-myc brings its expression under the influence of the promoter and/or enhancer within the retroviral LTR. In contrast, in most Burkitt lymphomas and murine plasmacytomas, activation is achieved by translocation to the immunoglobulin heavy chain locus. The role of the immunoglobulin locus in the activation is poorly understood. We have sought to directly demonstrate in vivo the efficacy of c-myc in lymphoid tumour induction. Transgenic mice were constructed bearing different forms of the c-myc gene and monitored for tumour development. Our results dramatically demonstrate that subjugation of c-myc to immunoglobulin enhancers converts this proto-oncogene into a potent leukemogenic agent for B lymphoid cells[1]. Since the predisposition is heritable, the lines of transgenic mice we have established provide a unique opportunity for analysis of the pre-neoplastic state.

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A1 STRUCTURE AND FUNCTION OF THE NEU ONCOGENE AND ITS ENCODED PROTEIN p185, Robert A. Weinberg, C.I. Bargmann, D.F. Stern, A.L. Schechter, M.-C. Hung, J.Drebin, and M.I. Greene, Whitehead Institute and Dept. of Biology, M.I.T. Cambridge, MA 02142, Dept. of Pathology, Harvard Medical School, Boston, MA 02115, Dept. of Medicine Tufts University School of Medicine, Boston, MA.. 02111

The neu oncogene has been detected by transfection of rat neuroblastoma DNAs into NIH3T3 cells. These tumors were induced by transplacental mutagenesis of rat embryos. Cells transfected with this oncogene display a novel cell surface antigen p185. The neu oncogene and normal gene have now been isolated in both genomic and cDNA forms. Its sequence reveals a homology with erbB and thus p185 bears structural homology with the EGF receptor. The oncogene-encoded p185 induces transformation even when expressed at low levels. In contrast, the p185 encoded by the normal protein has no effect on cell phenotype in vitro, even when expressed at high level. This suggests that the two genes differ in their sequences encoding protein structure. Analysis of the normal and oncogenic alleles shows no gross difference in structure. The lesion responsible for activation is being localized. Use of anti-p185 monoclonal anitbody also allows manipulation of the growth properties of neu transformed cells and this will also be descirbed.

Oncogene Products and Functions

A2 MULTIPLE 5'-EXONS ON C-ABL RNA SPECIES THAT COULD ENCODE MULTIPLE C-ABL PROTEINS, David Baltimore, Andre Bernards, George Daley and Yinon Ben-Neriah, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142 and Massachusetts Institute of Technology, Cambridge, MA 02139

Abelson virus contains the v-abl oncogene part of which encodes a protein-tyrosine kinase activity. Using v-abl as a probe, c-abl cDNAs were isolated from a pre-B-lymphoid cell line, 70Z/3. Four different cDNAs have been identified. The predicted proteins differ at their N-terminal ends but have common bodies. The common sequence includes most of the protein: from 20-45 N-terminal amino acids are variable and show no homology to each other or to any other proteins in Genbank. One N-terminus has a met-gly-gln structure that could be processed to N-terminal gly and then myristylated; the others have no gly near their N-termini. The upstream exon structure of one of the mouse c-abl clones is identical to published human c-abl cDNA.

The genomic organization that leads to the 4 c-abl transcripts is becoming clear. There are two pairs of potential 5' exons located separately fron one another. One pair remains to be cloned from the genome but appears to be found together in a single RNA transcript that is alternatively spliced. The second pair are near each other in the genome, are found in a single transcript and, at least in human DNA, appear to be located about 14 kb upstream of the common exon to which all 4 5'-exons are spliced. The bcr is similarly spliced to this same exon, as is evident from published data.

We have made anti-peptide sera to the N-terminal sequences of some of the predicted c-abl proteins. Thus far, one of these sera precipitates a c-abl protein. This same serum appears to precipitate the Philadelphia chromosome-related 210,000 molecular weight protein (in collaborative experiments with Owen Witte and Anne Marie Mes-Masson). Such a result is inconsistent with the proposed origin of this protein as a bcr-abl fusion at the common exon. This discrepancy is under study.

A3 STRUCTURE AND FUNCTION OF THE PRODUCTS OF VIRAL AND CELLULAR src GENES, Hidesaburo Hanafusa, Richard Jove, Joan Levy, Bruce Mayer, Hideo Iba, Fred Cross, David Pellman, Ellen Garber, Tatsuo Takeya, Carla Grandori, and Teruko Hanafusa, The Rockefeller University, New York, New York 10021

The src gene product of Rous sarcoma virus (RSV), $p60^{v\text{-}src}$, consists of three domains. The tyrosine kinase activity of this protein, which is essential for cell transformation, is mapped to the C-terminal half of about 30 kd. Mutations in most temperature-sensitive mutants can be mapped in this domain. The first 14 amino acids are required for myristylation of the N-terminus of this protein, which in turn is required for membrane association and cell transformation, even though mutations in this region do not affect the protein kinase activity. In addition, deletion mapping showed that the remaining N-terminal half of $p60^{v\text{-}src}$ has an influence on the transformation phenotypes of cells.

RSV variants in which v-src is replaced by c-src are inactive in both morphological transformation of fibroblasts and stimulation of growth of neuroretinal cells, even though they direct the synthesis of $p60^{c\text{-}src}$ in amounts comparable to that of $p60^{v\text{-}src}$ in RSV-transformed cells. These restricted biological properties of $p60^{c\text{-}src}$ appear to correlate with its restricted protein kinase activity. We have analyzed the structures of various transforming $p60^{src}$ proteins that have been derived from $p60^{c\text{-}src}$ by spontaneous mutation. In two spontaneous transforming viruses we found that each has a single point mutation in the kinase domain within the src gene. The substitution of various portions of $p60^{v\text{-}src}$ for the corresponding parts of $p60^{c\text{-}src}$ by in vitro recombination, including one amino acid in the kinase domain in one construct and three amino acids in the first one-fifth of N-terminal region in another construct, converted $p60^{c\text{-}src}$ to transforming proteins. In every case, activation of the transforming potential is associated with an increase in the level of protein kinase activity. These results indicate that a single mutation can activate the $p60^{c\text{-}src}$ kinase and that changes in the N-terminal structure can also activate the kinase. Some mutants were found to be able to stimulate the sustained growth of neuroretinal cells without inducing morphological transformation or colony formation in fibroblasts, but the converse was never found, suggesting that the latter phenotypes require additional changes in infected cells.

A4 VIRALLY INDUCED HEMATOPOIETIC CELL TRANSFORMATION: MULTI-STEP LEUKEMOGENESIS AND GROWTH FACTOR INDEPENDENT CELL PROLIFERATION. Allen Oliff and Steven M. Anderson, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.
Friend murine leukemia virus (F-MuLV) is a replication competent, type C, retrovirus that causes erythroid, myeloid and lymphoid leukemias in susceptible strain of mice. F-MuLV induced erythroid and myeloid leukemias are characterized by hepatosplenomegaly, anemia and a rapidly fatal disease course. Typically, 90-100% of inoculated animals develop leukemia within 6-8 weeks, and >90% of mice die within 3-4 months. Despite the extreme virulence of these leukemias, the hematopoietic tissues obtained from leukemic mice do not propagate in cell culture and do not form tumors in syngeneic mice. However, rare ($<1/10^6$) transplantable leukemia cells can be isolated from diseased mice that survive beyond 14 weeks. To identify the physiologic and genetic basis for the differences between these leukemia cell populations we tested the growth factor responses and measured the levels of oncogene expression in: transplantable murine leukemia cells, non-transplantable leukemia cells, and normal mouse bone marrow. In the absence of exogenous growth factors, only the transplantable leukemia cells exhibit continuous growth in cell culture. In the presence of WEHI-3 cell conditioned media or purified interleukin-3, 100% of the non-transplantable leukemia cell explants yield immortal cell lines in vitro. These growth factor dependent, non-transplantable cell lines exhibit the same growth kinetics as the transplantable leukemia cells. No immortal cell cultures were established from normal bone marrow cells with or without conditioned media. No single oncogene or growth factor mRNA was clearly more abundant in the leukemia cell populations versus the normal bone marrow cells. Nonetheless, introduction of specific oncogenes (abl or src) into the non-transplantable leukemia cells converts these cells into tumorigenic, growth factor independent cell lines. Ki-ras, Ha-ras, fos and myc do not alter the growth factor dependence or tumorigenicty of the non-transplantable leukemia cells. None of these oncogenes including abl or src altered the in vitro growth characteristics of the normal bone marrow cells using our culture techniques. We conclude that at least two genetic events are needed to convert murine hematopoietic precursors into tumorigenic cell lines. The first event permits immortal cell growth in the presence of specific growth factors. The second event abrogates the growth factor dependence of the leukemia cells resulting in tumorigenic cell growth.

A5 MICROINJECTION STUDIES OF CELLULAR ONCOGENE FUNCTION, Dennis W. Stacey, Mark R. Smith and Steven J. DeGudicibus, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

We have microinjected monoclonal antibodies (Furth *et al.*, J. Virol. 43:294, 1982) against viral *ras* p21 proteins in an effort to determine the biological function of cellular *ras* (c-*ras*) proteins. Immunoglobulin from monoclonal 259 clearly neutralized the activity of *ras* proteins within injected cells. This was the case with p21 protein synthesized by bacterial cells and purified as well as with *ras* proteins synthesized by the injected cell. When microinjected into NIH3T3 cells the neutralizing antibody efficiently blocked the initiation of DNA synthesis. Inhibition was observed up until just prior to the beginning of cellular S-phase but did not affect a cycle of DNA synthesis once initiated.

A variety of normal mouse and human fibroblast cell types along with mouse keratinocytes in primary culture were analyzed as above. In each case the primary cell types were reduced 90-95% in proliferation by the antibody. In contrast, several human tumor lines were not inhibited by the antibody. This was the case with most sarcoma and carcinoma lines tested except those containing a mutant, transforming c-*ras* gene. As evidence that the mutant *ras* gene did participate in proliferation, each tumor line with such a gene was reduced 60-95% in proliferation by injected antibody. These observations suggest c-*ras* proteins are involved in the control of proliferation in normal cells. In many tumor cells, however, the loss of control over proliferation correlates with a loss of the involvement of c-*ras* proteins in the initiation of S-phase. Analysis of additional cell types along with NIH3T3 cells transformed by various retroviral oncogenes was undertaken to analyze this hypothesis.

Growth Factors and Receptors

A6 THE EGF-RECEPTOR KINASE: STRUCTURE, EVOLUTION AND PROPERTIES OF VARIOUS RECEPTOR MUTANTS, J. Schlessinger, Department of Chemical Immunology, The Weizmann Institute of science, Rehovot 76100, Israel.

The EGF-receptor is a 170 KD membrane glycoprotein which has 3 major functional domains, a large extra-cellular, glycosylated EGF-binding domain, a single hydrophobic trans-membrane region and a cytoplasmic kinase domain (1). The extracellular domain of EGF-receptor contains 2 cysteine rich clusters which reveal internal homology and repetition of the cystein residues. The Drosophila EGF-receptor homolog (2) contains 3 cysteine rich clusters. A novel receptor related to EGF-receptor, probably the human counterpart of the rat *neu* oncogene product (3), also has 2 cysteine rich clusters. The insulin binding domain of the insulin receptor contains a single cystein rich domain. From comparison of sequences of the extracellular domains of these receptors it is proposed that an ancestral receptor gene was formed by fusion of genes coding for a tyrosine kinase domain and an extracellular cysteine rich cluster. This ancestral gene evolved to form the insulin-receptor and, after duplication, evolved to form the EGF-receptor gene family. In Drosophila, gene triplication occurred to yield a receptor with three cysteine rich clusters.

Questions concerning the mechanism of action and regulation of EGF-receptor were addressed by exploring properties and cellular effects of various EGF-receptor mutants introduced into cultured cell lines. Transient expression of intact EGF-receptor and various EGF-receptor mutants in COS-1 cells was achieved by using shuttle vector containing the SV-40 origin of replication. The same vector together with the DHFR gene were used to obtain stable cell lines expressing different amounts of EGF-receptor in CHO cells, which are normally devoid of EGF-receptor and its various mutants. A retroviral shuttle vector was used to express intact EGF-receptor and EGF-receptor mutants in mouse $\psi 2$ cells and for obtaining retroviruses containing sequences coding for the intact receptor and its various mutants. Initially we have generated constructs with deletions in the cytoplasmic domain of the EGF-receptor including a receptor mutant which has only 8 amino acids in the cytoplasmic domain (devoid of Thr 654). We also have introduced specific linkers into different restriction sites along the full size cDNA of EGF-receptor. Using this approach we have explored the role of various receptor domains in the regulation of receptor internalization, endocytosis and transformation.

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2. E. Livne, et al., Cell 40:599-607 (1985).
3. A.L. Shechter, et al. Nature 312:513 (1984).

A7 PLATELET-DERIVED GROWTH FACTOR AND DOUBLE STRANDED RIBONUCLEIC ACIDS STIMULATE EXPRESSION OF THE SAME GENES IN 3T3 CELLS, Charles D. Stiles, John N. Zullo, Brent H. Cochran, Alice S. Huang, Department of Microbiology and Molecular Genetics, Harvard Medical School, Children's Hospital and the Dana-Farber Cancer Institute, Boston, MA 02115

Platelet-derived growth factor (PDGF) stimulates expression of a "competence" gene family in Balb/c-3T3 cells. The c-*myc* and c-*fos* genes are contained within the competence family together with several functionally uncharacterized competence genes (JE, KC and r-*fos*) which have been isolated as cDNA clones. Here we show that double stranded ribonucleic acid is a potent inducer of the competence gene family. Infection with vesicular stomatitis virus (VSV) also induces expression of this gene family. Conversely, PDGF stimulates expression of genes which have been hitherto characterized as responsive to double stranded ribonucleic acids. These include the beta-fibroblast interferon and (2'-5')-oligoadenylate synthetase genes. It is conceivable that these PDGF-inducible genes function in a feedback loop to control 3T3 cell growth. Some of the genes such as c-*fos* and c-*myc* are induced quickly by PDGF (within 15-45 minutes) and may function to initiate a round of cell division. Other genes such as beta-fibroblast interferon and (2'-5')-oligoadenylate synthetase show a slower time course of induction and may function as feedback inhibitors of the growth response to PDGF.

Tumors and Differentiation

A8 STRUCTURE, FUNCTION, AND REGULATED EXPRESSION OF MYC-FAMILY GENES

Frederick W. Alt, Kathy Zimmerman, Nancy Kohl, George Yancopoulos, Ron DePinho, Edith LeGouy, and Perry Nisen, Department of Biochemistry and Molecular Biophysics, College of Physicians and Surgeons of Columbia University, New York, New York, 10032

The myc family of cellular oncogenes contains three well-defined members, c-myc, N-myc, and L-myc; our recent studies suggest that this family contains multiple additional members. Previously, we demonstrated that the N-myc and c-myc gene had a similar oncogenic potential in an *in vitro* transformation assay. More recently, we have determined that the N-myc gene has a number of striking structural similarities and differences with the c-myc gene including: (1) the overall structure and organization of two myc genes and their transcripts are very similar in that each gene contains 3 exons of which the first encodes a long 5' untranslated leader sequence. (2) the coding regions of the N- and c-myc genes share regions of substantial homology. (3) As with c-myc, extensive nucleotide sequence homology exists between the untranslated regions of the human and murine N-myc transcripts; however, the N-myc and c-myc untranslated regions are totally divergent. (4) the N-myc transcriptional promoter differs from that of c-myc and is more related to the promoter of the SV40 virus.

Despite apparent similarities in structure and function, we have demonstrated that high level expression of N- and L-myc genes is very restricted with respect to tissue and developmental stage while that of c-myc is more generalized; the unique expression patterns of the myc genes in normal cells generally predicts the types of tumors in which they are expressed. We find the highest levels of N- and L-myc expression in developing neural tissues. However, the N-myc gene appears to have a role in the early stages of multiple differentiation pathways; this property is most clearly evidenced by N-myc expression patterns in differentiating B-lymphoid cells. Our intial finding of high level N-myc expression in fetal kidney led to our more recent demonstration of high level N-myc expression in most Wilm's tumors (which derive from embryonic kidney cells). Activated N-myc expression may be a characteristic of a variety of tumors which derive from primitive normal cell precursors, including a set of childhood embryonic tumors which may have a genetic component to their etiology (retinoblastoma, wilm's tumor, neuroblastoma, and medulloblastoma). Our initial transfection experiments suggest that sequences which confer tissue or stage-specfic patterns of N-myc expression are located within or near the N-myc gene. In addition, we find that c-myc expression is down-regulated in cells which express elevated levels of N-myc. Together, our findings suggest that differential myc-family gene expression could play an important role in normal differentiation processes.

A9 MOLECULAR GENETICS OF HUMAN B AND T CELL NEOPLASIA, Carlo M. Croce, The Wistar Institute, 36th at Spruce Street, Philadelphia, PA 19104

It is clear that specific chromosomal translocations are responsible for the majority of human B cell neoplastic diseases. These translocations place proto-oncogenes in close proximity to genetic elements capable of activating gene transcription *in cis* along considerable chromosomal distances. Thus the juxtaposition of the proto-oncogenes and immunoglobulin loci result in the deregulation of the involved proto-oncogene that is transcribed constitutively at elevated levels leading to neoplasia. Interestingly, the molecular mechanisms involved in the t(11;14) and t(14;18) chromosome translocations seem to involve the physiologic enzymatic system responsible for V-D-J joining. These translocations do not occur at random, but are the result of mistakes in V-D-J joining, where the V-D-J joining enzyme joins separated segments of DNA on two different chromosomes instead of joining two separated segments of DNA on the same chromosome on the basis of signal sequences for V-D-J joining. In view of these findings it seems logical to speculate that the enzymes involved in immunoglobulin gene rearrangements may also be involved in the t(8;14), t(2;8) and t(8;22) chromosome translocations observed in Burkitt lymphomas.

Interestingly, a similar situation seems to occur in T cell neoplasms. The molecular analysis of T cell tumors indicates an important role for the locus of the alpha chain of the T cell receptor in the pathogenesis of these diseases. Since the locus for the alpha chain of the T cell receptor is split by the chromosome break at band 14q11.2 and since the Cα locus translocates either to a different chromosome or to region 14q32, it is easy to predict that by "chromosome walking" it will be possible to clone and identify the genes that are involved in the pathogenesis of most T cell tumors.

Since most of human T cell malignancies in areas endemic for human T cell leukemia virus 1 (HTLV-1) also carry either translocations or inversions involving chromosome band 14q11.2, it seems likely that HTLV-1 *per se* may not be a leukemogenic agent and might have an indirect role similar to that of EBV in the pathogenesis of African Burkitt lymphoma. Thus infection with HTLV-1 may increase the number of T cells susceptible to developing a chromosome translocation, possibly during T cell receptor gene rearrangements.

In conclusion, the study of the molecular genetics of specific chromosome translocations in human B and T tumors is providing a detailed explanation of the mechanisms and genes involved in these neoplasms.

A10 COOPERATIVITY OF VIRAL ONCOGENES IN AVIAN ERYTHROID CELLS, T.Graf, P.Kahn, K.Damm, I.J.Stanley, L.Frykberg, B.Vennström and H.Beug, Differentiation Programme, EMBL - European Molecular Biology Laboratory, Meyerhofstrasse 1, 6900 Heidelberg, West-Germany.

The ES4 strain of AEV is a chicken retrovirus which carries 2 oncogenes and transforms erythroblasts and fibroblasts in vivo and in vitro. V-erbA by itself is nontransforming while v-erbB transforms both erythroblasts and fibroblasts (1). Erythroblasts transformed with v-erbB only differ from v-erbA + v-erbB erythroblasts in that they are still capable of spontaneous differentiation and exhibit complex growth requirements similar to normal erythroid progenitor cells (2). To investigate the mechanism of cooperativity between these two oncogenes we examined td359 AEV, a mutant which has lost its erythroid transforming capacity but still transforms fibroblasts (3); and r12 AEV, a revertant recovered from animals infected with td359 AEV and which has regained full erythroid transforming capacity. Molecular analysis of the two mutants and of viral constructs containing mutant and wild type v-erbA and v-erbB oncogenes in various combinations showed that a) td359 carries separate deletions in both oncogenes and its v-erbA gene is nonfunctional (tested in combination with wild type v-erbB), b) v-erbA from wild type virus can restore the erythroid transforming capacity of v-erbB from td359, and c) the v-erbB gene of r12 AEV is still defective; however, its v-erbA gene is even better in compensating for the defective v-erbB gene than is v-erbA from wild type virus. We are currently trying to identify the alterations in this virus that led to a "super erbA".
To study whether v-erbA cooperates with oncogenes other than v-erbB, erythroid cells transformed by the tyrosine kinase oncogenes v-src, v-fps and S13 virus oncogene (4,5) as well as by v-Ha-ras were superinfected with a v-erbA encoding virus. In all cases, the cells acquired a phenotype similar to v-erbA, v-erbB cells. A construct containing both v-src and v-erbA not only induced fully transformed erythroid cells in vitro but also caused acute erythroleukemias (in addition to sarcomas) in vivo. Our results thus show that 1.) the v-erbA protein can cooperate with several kinase- and a non-kinase encoding oncogene product (suggesting that it does not directly interact with v-erbB protein); 2.) it can compensate for defects in v-erbB; and 3.) it can modulate the in vivo transforming specificity of a sarcoma-inducing oncogene.

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A11 PROTO-ONCOGENE ~~c-~~: ROLE IN DEVELOPMENT, GROWTH AND DIFFERENTIATION, Inder M. Verma, The Salk Institute, San Diego, California 92138.

Proto-oncogenes are expressed in a stage and tissue-specific manner during the prenatal development of the mouse. Some proto-oncogenes like c-fos and c-fms are expressed at the highest levels in extraembryonal tissues, while others like c-abl accumulate at high levels during mid-gestation (day 10-11). Homologues of c-ras^{Ha} are expressed at all stages of prenatal development, while transcripts of oncogene mos cannot be detected at any stage. Such differential expression is indicative of the role during development. Tissue-specific expression was also detected in postnatal tissues.

When quiescent fibroblastic cells are stimulated with growth factor or mitogens, both c-fos and c-myc genes are expressed transiently. Expression of the c-fos gene precedes that of c-myc; maximum induction occurs in 20-30 minutes after stimulation, and expression is shut off by 120 minutes. The c-fos protein, which is extensively modified and nuclear is location, is expressed transiently. Transient expression of the c-fos gene is also observed when a rat pheochromocytoma cell line (PC12) is induced to differentiate in response to either nerve growth factor (NGF), cyclic AMP or K^+, but not upon addition of dexamethasone. Rapid and transient expression of c-fos gene is also observed following hepatectomy or stimulation of T and B-cells with mitogens.

Rapid expression of the c-fos gene transcripts is observed when monocytic or monomyelocytic cell lines are induced to differentiate into macrophages. No c-fos expression can be witnessed when monomyelocytic cells differentiate to granulocytic lineage. A 20-fold induction of c-fos mRNA occurs within 30 minutes of addition of the inducer TPA, which decreases by 4-5 fold by 60 minutes and remains constitutive for the next 96-105 hours when 99% of the monocytes differentiate to adhering mature macrophages. However, c-fos protein is synthesized only for 60-120 minutes. Since sustained amounts of c-fos protein can induce transformation, the cell has devised a mechanism to shut off its synthesis without termination of transcripts. The implication of these findings in relation to the role of proto-oncogenes during normal metabolic processes of a cell will be discussed.

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Phenotypic, Karyotypic and Genetic Heterogeneity of Tumors

A12 GENETIC EVENTS UNMASK RECESSIVE ALLELES TO INITIATE MALIGNANCY, Brenda L. Gallie and Ron G. Worton, Hospital for Sick Children, University of Toronto, Toronto, Canada M5G 1X8.

In cells that are heterozygous for a recessive genetic marker, expression of the recessive phenotype depends on loss of the dominant wild-type allele. In early studies with Chinese hampster hybrid cells that were heterozygous for recessive drug resistance genes, expression of the recessive phenotype (drug resistance) was shown to occur by several mechanisms: wild-type gene inactivation, chromosome loss with duplication of the homologue carrying the recessive marker and probably mitotic recombination (1).

The chromosomal locations of the genes responsible for predisposition to the childhood malignancies retinoblastoma (RB) and Wilm's tumor are known by linkage studies with chromosomal deletions and enzyme markers. Studies of such tumor cells in comparison to the normal constitutional cells of the patients, using restriction fragment length polymorphic (RFLP) markers near the predisposing genes, has shown that these genes are recessive to the normal wild-type allele at the cellular level (2,3). Expression of the recessive phenotype (malignancy) results from the same genetic events that were observed in the hybrid cells: the wild-type allele is lost by mitotic recombination or chromosome loss with duplication of the mutant chromosome in 70% of cases; wild-type gene inactivation may initiate malignancy in the remaining 30% of cases (4).

Although every cell of the individuals with germ line heterozygous mutations for these tumors is at risk to develop homozygosity for the recessive mutation, malignancy occurs only in a few specific tissues. Survivors of RB are at risk to later develop other tumors, particularly osteogenic sarcomas. Studies with RFLP markers show that these second primary tumors also result from unmasking of the recessive phenotype by loss of the normal allele at the RB locus (5).

Further chromosomal rearrangements and amplification of "oncogenes" are late genetic events in these "homozygous" tumors (6). In other malignancies, such as neuroblastoma, in which studies have so far focused on oncogene amplification and chromosome rearrangements, unmasked recessive mutations may also be the critical initiating events.

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A13 MULTIDRUG RESISTANCE, I. Pastan, D.-w. Shen, M. Cornwell, T. Fojo, N. Richert, K. Ueda, D. P. Clark, I. Roninson* and M. M. Gottesman, Laboratory of Molecular Biology, NCI, NIH, Bethesda, MD 20892 *Center for Genetics, Univ. of Illinois College of Medicine at Chicago, Chicago, IL 60612

To study the clinically important problem of development by tumors of simultaneous resistance to multiple chemotherapeutic agents, we have established three multidrug resistant human KB carcinoma cell lines in culture. These cell lines, selected independently for resistance to colchicine, Adriamycin or vinblastine, are cross-resistant to the selecting agents as well as to a wide variety of other hydrophobic drugs which differ in their mechanisms of toxicity (1). Drug accumulation in these cells is decreased dramatically compared to their parent KB cell line or revertant cells(2). Mixed vesicles prepared from the resistant cells accumulate 8-fold more vinblastine than vesicles prepared from parental or revertant cells. The development of this in vitro system, which reflects the differences in drug accumulation between sensitive and resistant lines, should allow us to identify cellular components responsible for this altered accumulation. All of our most resistant multidrug resistant cell lines express amplified sequences, known as mdr1. Human mdr1 sequences were cloned in two ways: (1) Directly out of multidrug resistant human cells using the technique of in gel renaturation (3) or (2) By using a cloned probe which detects amplified sequences in multidrug resistant Chinese hamster cell lines (4,5). Resistant cell lines express levels of a 4.5 kb mRNA encoded by the mdr1 gene which are proportional to their degree of multidrug resistance. Expression of this 4.5 kb mRNA preceeds gene amplification during the development of multidrug resistance in the KB cell lines. Using DNA derived from a KB multidrug resistant cell line, we have transferred the multidrug resistance phenotype to sensitive mouse NIH 3T3 cells. Human mdr1 sequences which are expressed at high levels are cotransferred with the multidrug resistance phenotype in both primary and secondary transformants, indicating that expression of the mdr1 gene is tightly linked to the development of multidrug resistance.

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A14 DNA REARRANGEMENT, RELOCATION AND AMPLIFICATION IN CELL LINES AND PRIMARY TUMORS OF NEUROECTODERMAL ORIGIN, Samuel Latt(1,2), Yossef Shiloh(1), Kazuo Sakai(1), Elise Rose(1), Garrett Brodeur(3), Tim Donlon(1), Bruce Korf(1), Michael Hearlein(1), John Kang(1), Helene Stroh(1), Peter Harris(1), Gail Bruns(1), and Robert Seeger(4,5); Departments of Pediatrics(1) and Genetics(2), Harvard Medical School, Boston, Mass. 02115, and Washington University School of Medicine, St. Louis, Mo. 63110(3), and the Department of Pediatrics, UCLA School of Medicine(4) and the Children's Cancer Study Group(5), Los Angeles, Calif. 90024.

DNA amplification in human neuroblastoma and retinoblastoma lines, as manifested by either homomgeneously staining regions(HSRs) or double minute bodies(DMs), is accompanied by DNA relocation and, in some cases, interesting DNA rearrangement. Extensive amplification of the oncogene N-myc, and to a systematically lesser extent, other DNA fragments amplified in neuroblastoma cell lines, is found in advanced stage neuroblastomas. The heirarchy with which DNA fragments are amplified in primary neuroblastomas, and perhaps neuroblastoma cell lines as well, contains information about the relative ordering of DNA fragments around N-myc in differently sized amplification units in these cells. Experiments utilizing somatic hybrid DNA panels, DNA dosage blotting, and in situ hybridization map those DNA fragments which are extensively amplified in neuroblastoma cells to the tip of the human chromosome #2 short arm, near band 2p24. In contrast, some DNA fragments amplified in the neuroblastoma line IMR-32, but not in primary tumors, map either to the middle of 2p(2p15-2p16) or the proximal part(near band 2p13) of this chromosome arm. Evidence thus far is compatible with a molecular reorganization, possibly extrachromosomal, and splicing of DNA from these three widely spaced regions of human 2p into amplification units contained in the HSRs of IMR-32 cells. In other neuroblastoma cell lines and primary tumors, DNA amplification is associated with extensive rearrangement. For one particular amplified DNA fragment, this rearrangement occurs at a specific point, bordered by DNA rich in A-T base pairs. In one neuroblastoma cell line, NB-9, this rearrangement does not reflect a simple DNA recombination. Instead, a complex, at least three-component DNA splice event has occurred. The mechanisms underlying the unusual DNA recruitment, rearrangement, and amplification in these neuroectodermal tumors and cell lines should prove of basic interest in understanding the dynamics with which the human genome changes. Moreover, the consequences of these events may be of clinical diagnostic and prognostic value.

A15 SOMATIC MUTATION IN THE Ig V_H GENE OF A B-CELL LYMPHOMA, R. Levy, S. Levy, M. Cleary, T.C. Meeker, E. Lee, M. Trela, J. Sklar. Department of Medicine, Stanford University, Stanford, CA 94305

We have reported that the tumor cells of some patients with monoclonal B cell lymphoma exhibit idiotypic heterogeneity that can be detected by monoclonal antibodies (NEJM 312:1658, 1985). To understand this idiotypic heterogeneity at the nucleic acid and protein sequence level we carried out genomic and cDNA cloning to isolate the heavy chain V region (V_H) genes expressed by an IgM positive lymphoma. Eight V_H nucleic acid sequences, each representing the gene expressed by a different cell of the tumor, were obtained and the amino acid sequences were deduced. It is clear from an examination of the nucleic acid sequences that they were all derived from members of a single clone of cells. However, no two sequences were identical. The two most divergent isolates differed by 14/357 nucleotides and by 8/119 amino acids, while the two most similar differed by 6/357 nucleotides and 1/119 amino acids. Appropriate controls indicated that this variation was present in the tumor population and was not due to cloning or sequencing artifacts. One consequence of these differences is the alteration of a glycosylation site (Asn-X-Ser) resulting in variation in heavy chain molecular weight. In this patient we were able to conclude that our anti idiotype antibody recognized a heavy chain epitope that included amino acid 54. While nucleic acid substitutions were found throughout the V region, they were especially prevalent in CDR2 and in one site within FR3. No mutations were found within CDR1 or CDR3. Within CDR2 the ratio of amino acid replacement to silent mutations was 8.5, a value which would be expected from random mutation, whereas outside of CDR2 this ratio was 0.55. This observation raises the possibility that antigen or idiotype networks might be exerting selective pressure to conserve the structure of part of the Ig molecule including CDR1 and CDR3, as occurs in normal B cells during an immune response.

Tumor Progression, Metastasis and Resistance to Drug Therapy

A16 P-GLYCOPROTEIN AND MULTIDRUG RESISTANCE, Victor Ling, The Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario, Canada M4X 1K9

One reason for failure in chemotherapeutic treatment of advanced cancers may be the outgrowth of multidrug-resistant tumor cells. This phenotype of resistance to unrelated drugs is frequently observed in human and animal cell lines selected for resistance to a single chemotherapeutic agent. A feature in common among a wide variety of multidrug-resistant lines is the increased expression of a 170,000 dalton transmembrane surface glycoprotein (P-glycoprotein). In most cases, this results from amplification of the P-glycoprotein genes. Overexpression of P-glycoprotein likely plays a direct role in mediating the reduced drug accumulation characteristic of multidrug-resistant lines. Monoclonal antibodies specific for different epitopes of P-glycoprotein have been isolated. Such reagents have been used to detect the expression of P-glycoprotein in animal tumors resistant to chemotherapy, and in biopsy samples from cancer patients. These studies indicate that multidrug-resistant tumor cells can arise with a high frequency and that such cells may present a major obstacle to successful chemotherapy. Supported by NCI of Canada, OCTRF, and NIH of USA.

A17 DIFFERENTIAL GENE AND GLYCOPROTEIN EXPRESSION BETWEEN LOW AND HIGH METASTATIC SUBLINES OF MURINE LARGE CELL LYMPHOMA. G. L. Nicolson, R. A. LaBiche, M. Frazier, R. J. Tressler and T. Irimura, Depts. of Tumor Biology and Medical Oncology, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030, and Graduate School of Biomedical Sciences, University of Texas Health Science Center, Houston, TX 77030.

Metastatic variants of RAW117 large cell lymphoma have been sequentially selected in vivo for liver metastasis or in vitro for loss of lectin-binding sites. Analysis of sialoglycoproteins (sgp) indicates increased expression of WGA-binding sgp on the more metastatic cells. In particular, the expression of a M_r ~150,000 sgp correlates with liver metastasis. In addition, highly metastatic cells lose MoMuLV surface and internal components, such as gp70, p30 and p15 (Reading et al., PNAS 77:5943, 1980). To examine differential gene expression we have established a cDNA library of 17,600 clones from poly A^+, mRNA isolated from the highly metastatic RAW117-H10 line. The cDNA library was screened by exposure of replicate filters containing the library to ^{32}P-cDNA probes made from both RAW117-P and -H10 sublines. Approximately 93% of the cDNA clones yielded detectable signals by computer-assisted densiometric scanning. Of the cDNAs detectable by computer-assisted scanning, 99.5% of these were identical between P and H10 sublines by data regression analysis. Approximately 100 out of 17,600 cDNA clones were differentially expressed; about 1/2 of these were MoMuLV transcripts determined by probing with MoMuLV cDNA. The remaining cDNA clones were expressed either in significantly lower or higher amounts in the highly metastatic H10 subline. The results indicate that there are unique differences between low and high metastatic RAW117 cells, and that only a few genes are differentially expressed in the highly metastatic variant cells.

Supported by NCI grant RO1 CA29571 to G.L. Nicolson.

A18 MDR GENE AMPLIFICATION IN MULTIDRUG-RESISTANT HUMAN CELLS, I.B. Roninson, J.E. Chin and K. Choi, Center for Genetics, University of Illinois College of Medicine, Chicago, IL 60612.

The ability of tumor cells to develop simultaneous resistance to multiple cytotoxic drugs constitutes a major problem in cancer chemotherapy. Multidrug resistance in mammalian cell lines results from decreased intracellular drug accumulation, apparently as a result of alterations in the plasma membrane. In the previous studies, two independently derived multidrug-resistant sublines of Chinese hamster cells were found by the in-gel DNA renaturation assay to contain a common set of amplified DNA fragments (1). These commonly amplified sequences were cloned and shown to contain a transcribed DNA region, presently designated mdr, expression of which correlates with multidrug resistance in Chinese hamster cells (2). Different subclones of the hamster mdr gene were used as probes for hybridization with human DNA. One of the subclones, pDR4.7, cross-hybridizes with two different sequences in human DNA, designated mdr1 and mdr2. Either mdr1 alone or both mdr1 and mdr2 are amplified in multidrug-resistant sublines of human KB cells selected by the group of M. Gottesman and I. Pastan at NIH for a high degree of resistance to colchicine, vinblastine or Adriamycin. In some sublines gene amplification was accompanied by a specific rearrangement of mdr2. The amplified copies of mdr1 and mdr2 are lost in a subline of multidrug-resistant KB cells, which has reverted to a low degree of drug resistance. Fragments of the human mdr1 and mdr2 regions, cross-hybridizing with the hamster pDR4.7 clone, have been isolated and analyzed for the presence of transcribed sequences. mdr1, but not mdr2, was found to encode a 4.5 kb mRNA, which was expressed in the multidrug-resistant sublines but not in the parental KB cells. As described in the accompanying abstract of Pastan et al., the development of multidrug resistance in KB cells is initially accompanied by elevated expression of mdr1 mRNA without amplification of the genomic sequences. Subsequently, expression of this mRNA is increased simultaneously with amplification of mdr1 DNA. Increased expression and amplification of mdr1 were also found in multidrug-resistant sublines of human leukemia and ovarian carcinoma cells. Additional evidence for the role of mdr1 in the development of multidrug resistance comes from the finding that acquisition of the multidrug-resistant phenotype by mouse NIH 3T3 cells upon transfection with total genomic DNA from multidrug-resistant human cells correlates with the transfer of the human mdr1 gene (for details, see the abstract by Pastan et al.) These results suggest that activation and subsequent amplification of mdr1 is a common mechanism for multidrug resistance in human cells. The nature of the mdr1 gene product and its relationship to P-glycoprotein, a common marker of multidrug-resistant cell membranes (3), are presently being investigated.

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In Vitro/In Vivo Correlations of Diagnosis and Treatment of Tumors

A19 MOLECULAR GENETIC ANALYSIS OF NEUROECTODERMAL TUMORS, Mark A. Israel, Catherine McKeon, Lee Helman, Carol Thiele, and Orsolya Csuka, Pediatric Branch, National Cancer Institute, Bethesda, MD 20892, and Research Institute of Oncopathology, Budapest, Hungary.

The genetic analysis of human solid tumors has lead to an elucidation of the molecular alterations which characterize these tumors. We have developed strategies to utilize these insights in the clinical management of patients with these tumors. Towards this end, we have developed a profile of altered gene expression which corresponds to clinically defined subgroups of neuronal tumors. The results of these studies indicate that amongst histopathologically indistinguishable neuronal tumors there are clinically identifiable subgroups which have distinct patterns of oncogene expression. While members of the myc family of genes have been particuarly useful in this regard, the expression of c-myb and c-ets is also consistently altered in these tumors. An unexpected observation was the finding that in neuroepitheliomas, one of the neuronal tumors examined, we found the expression of oncogenes not located near the characteristic rcp(11;22) (q24;q12) to be consistently altered.

In addition to these already well defined proto-oncogene probes, we have utilized differential cDNA cloning to identify other molecular markers, which distinguish between closely related tumors of the peripheral nervous system. To date, the tumor we have best characterized is pheochromocytoma. We have isolated several cloned DNAs which recognize genes either uniquely expressed or expressed at much higher levels in pheochromocytoma than in normal neuronal issues, neuroblastoma, neuroepithelioma, and tumors of other tissues arising from the neural crest. These genes constitute the framework upon which a molecular fingerprint of pheochromocytoma can be constructed. Currently we are characterizing such fingerprints for neuroblastoma and neuroepithelioma as well as pheochromocytoma. We believe that such molecular markers may supplement the histologic classification of solid tumors in a manner analogous to the use of monoclonal antibodies in classifying tumors of the lymphoid system. Together with a characterization of the pattern of oncogenes activated in a particular tumor, it is likely that the utilization of such genetic markers will allow for both a more accurate diagnosis of closely related tumors and a more efficacious stratification of homogeneous disease entities so that their clinical behavior and therapeutic responsiveness can be more meaningfully assessed.

A20 MECHANISMS OF GROWTH REGULATION OF HUMAN BREAST CANCER IN VIVO AND IN VITRO, Marc Lippman, Robert Dickson, Edward Gelmann, Attan Kasid, Nancy Davidson, George Wilding, Sandra Swain, Susan Bates and Mary McManaway, Medicine Branch, National Cancer Institute, Bethesda, MD 20892

The mechanisms by which estrogens and antiestrogens regulate the growth of some human breast cancers and the pathways by which hormone independence occurs are incompletely understood. In this presentation work will be presented which supports four basic hypotheses. First, that estrogens directly interact with human breast cancer cells to alter gene expression at the level of gene transcription. Our progress in identifying genes induced as early as 4 hours after estrogen stimulation of hormone dependent human breast cancer cell lines will be described. Second, that amongst the gene products induced by estrogens are a group of separate secreted growth factor activities which are a able to directly stimulate tumor cells (paracrine effects) as well as stimulate surrounding stroma (autocrine effects). These growth factors are able to stimulate the growth of quiescent fibroblasts, stimulate the growth of cells already in the cell cycle, and reversibly induce acquisition of the malignant phenotype by non transformed cells. Growth factor activities which we have identified as secreted by these human breast cancers are Insulin-like growth factor I (IGF-I); transforming growth factor α (TGFα); platelet derived growth factor (PDGF); transforming growth factor (TGFβ); a novel, high molecular weight epithelial growth factor; and thymosin α_1, and β_4. Third, the acquisition of the hormone independent phenotype represents the constituitive secretion of identical growth factors at higher concentrations without hormonal regulation. Several lines of hormone independent breast cancer cells all secrete increased amounts of IGF-1, TGF's, and a high molecular weight epithelial growth factor. In addition, we will present work in which we have converted hormone dependent human breast cancer to a hormone independent phenotype by the introduction of an activated viral ras sequence from the Harvey sarcoma virus. These cells show increased production of IGF-I, TGFα, and TGFβ. Fourth, antiestrogens can inhibit the growth of hormone dependen breast cancer cells by the positive induction of RNA and protein products. This may be responsible for some or all of the induced inhibitory effects of antiestrogens. Data will be supplied during the presentation to support all of these hypotheses. In addition we will present evidence that these pathways of hormonal regulation of tumor growth are relevant to in vivo tumor progression using a nude mouse model system as well as to in vitro growth stimulation.

Oncogene Identification, Activation, Products and Functions

A21 REGULATION OF N-MYC TRANSCRIPT NUMBER IN Y79 RETINOBLASTOMA CELLS. C. M. Amy, W.-H. Lee, and J. Bartholomew, Lawrence Berkeley Laboratory, Berkeley, CA 94720

The N-myc oncogene is amplified and/or expressed at a high level in many cell lines derived from neuronal tumors; non-neuronal cells apparently do not express this gene. Y79 cells derived from a retinoblastoma tumor have been shown to have 50 - 100 copies of the N-myc gene transcript per cell. We first tested whether N-myc is preferentially expressed during specific parts of the Y79 cell cycle to investigate whether N-myc might play a role in growth control. Y79 cells were synchronized with double thymidine blocks at the beginning of S, then released from the block and collected for isolation of total cytoplasmic RNA and for analysis by flow cytometry. N-myc DNA probes hybridized with dotblotted RNAs and RNAs from Northern blots of agarose-formaldehyde gels showed no differences in the number of transcripts as the cells traversed G_1 and entered the S phase over a period of 12 hrs. Protein synthesis inhibitors added to randomly growing Y79 cells did show a dramatic increase in N-myc transcript number over a period of 4 - 6 hrs; control genes showed no such increase. Inhibition of RNA synthesis by actinomycin D treatment led to a rapid decrease in N-myc transcript number which was partially prevented by protein synthesis inhibitors. Thus, N-myc transcripts have a short half-life whose stability may be increased by inhibition of protein synthesis. These results confirm that similarities between c-myc and N-myc go beyond their partial sequence homology and suggest that N-myc may serve the same (as yet unknown) function in neuronal cells that c-myc plays in non-neuronal cells.

A22 GUANINE NUCLEOTIDES AND RAS p21 PROTEINS MODULATE PHSPHORYLATION OF 36 AND 17 KD PROTEINS IN SUBCELLULAR FRACTIONS. Joseph M. Backer, and I. Bernard Weinstein, Comprehensive Cancer Center, Columbia University, N.Y., N.Y. 10032.

We have found that when membrane fractions isolated from rat liver mitochondria and other cell fractions, are incubated with [γ-^{32}P]ATP there is phosphorylation of 36 and 17KD proteins. These proteins together with their protein kinase(s) are released as a 200-250KD complex by incubation of the isolated membrane fractions at 20°C for 30 minutes with 10mM glucose-6-phosphate. Phosphorylation of these proteins is modulated by submicromolar concentrations of guanine nucleotides and by bacterially expressed ras p21 proteins. A normal p21 protein stimulates phosphorylation of the 36KD protein and inhibits phosphorylation of the 17KD protein. Two transforming p21 proteins inhibit phosphorylation of both the 36 and 17KD proteins. Effects of the transforming proteins on phosphorylation of the 36 and 17KD proteins depend on the structure of the guanine nucleotide associated with the ras p21 protein. These results suggest that ras p21 proteins may function by directly modulating the phosphorylation of specific membrane-associated proteins.

These studies were supported by NCI Grant CA21111 and the National Foundation for Cancer Research.

A23 CHEMICAL CARCINOGEN-INDUCED THYMIDINE KINASE GENE ACTIVATION IS ASSOCIATED WITH UPSTREAM GENE ALTERATIONS AND GENOMIC HYPOMETHYLATION. Frederic G. Barr, Sridharan Rajagopalan, Craig A. MacArthur, and Michael W. Lieberman. Department of Pathology, Fox Chase Cancer Center, Philadelphia, PA 19111.

We have analyzed the mechanism of activation of an inactive but functionally intact hamster thymidine kinase (TK) gene by chemical carcinogens. RJK92 cells, a bromodeoxyuridine-resistant Chinese hamster line with an azacytidine-activatable TK gene, were treated with N-methyl-N'-nitro-N-nitrosoguanidine. Aminopterin-resistant colonies appeared at a frequency 50-fold greater than in untreated controls. Greater than 80% of these resistant clones express detectable TK enzymatic activity; these TK^+ lines can be divided into low activity (75%) and high activity (25%) groups. Expression of detectable TK activity is absolutely correlated with demethylation of deoxycytidine residues in the 5' region of the gene. Four of the five high activity lines show extensive genomic hypomethylation which is associated with demethylation throughout the TK gene in all TK alleles. No structural changes in the TK gene were detected in the examined high activity TK^+ lines; however, analysis (with methylation-insensitive restriction enzymes) of fifteen low activity TK^+ lines revealed four instances of a structural alteration in the far upstream region of the TK gene and one instance of a low copy amplification of the gene. (Supported by NIH Grant CA39392).

A24 STRUCTURE AND DIFFERENTIAL EXPRESSION OF myb PROTO-ONCOGENE mRNA IN MURINE B-CELL TUMORS. Timothy P. Bender and W. Michael Keuhl. NMOB/NCI, Bethesda, MD. 20814.

We have examined the steady state levels of c-myb mRNA expression in a series of murine B-cell tumors. The pre B-cell tumors all express similiar high levels of c-myb mRNA while the B-cell lymphomas and plasmacytomas produce 20->250 fold lower levels. The 70Z/3.12 pre B-cell line makes a cytoplasmic mu H-chain and can be induced to transcribe kappa L chain. Upon induction with LPS >95% of the cells become surface IgM+. Concommitantly, levels of c-myb mRNA decrease by >90% and are in the range seen in mature B-cell lines. To further correlate c-myb mRNA expression with B-cell phenotype hybrid cell lines representing pre B-cell X B-cell and pre B-cell X plasma cells were made. In all cases, pre B-cell X plasma cell hybrids phenotypically resemble the more mature plasma cell phenotype in both Ig production and levels of c-myb mRNA production. By contrast, pre B-cell X B-cell hybrids phenotypically resemble the less differentiated pre B-cell phenotype in Ig, J-chain, Ia and c-myb mRNA production. Thus, steady state levels of c-myb mRNA correlates with the stage of B-cell developement. To initiate studies on the regulation of c-myb mRNA in B-cells we have isolated and sequenced two overlapping cDNA clones from a murine pre B-cell library which generate a composite sequence of 3413 bases of the c-myb mRNA. The sequence includes a single long open reading frame which, from the first Met codon, has coding capacity for 636 amino acids followed by a 1242 base 3' untranslated region. S1-nuclease protection studies with cDNA and genomic clones have demonstrated extreme heterogeneity at the 5' end of c-myb mRNA. The mechanism and biological relevance of this heterogeneity are being examined.

A25 CORRELATION BETWEEN INCIDENCE OF DMH(1,2-dimethylhydrazine)INDUCED COLON CARCINOMAS AND DNA DAMAGE IN FIVE GENETICALY DIFFERENT MOUSE STRAINS,Claudia Bolognesi and Lidia C. Boffa,Istituto Nazionale per la Ricerca sul Cancro,IST,Viale Benedetto XV n.10, 16167 Genova, ITALY.

Mice from strains with widely different susceptibilities to the colon-specific carcinogen DMH were tested for DNA damage in liver, kidney and colon after administration of the compound at a dosage that has been reported to induce high incidence of adenocarcinomas of the colon in rodents (50 mg/Kg,single administration). The strains studied were; AKR/J;DBA/2 totally resistant to the carcinogen, CD1 intermediately susceptible (60%) ,and SWR/J;C57 BL/6N very susceptible. This susceptibility follows Mendelian laws.Data are expressed as elution rate constant;($K\ ml^{-1} = \frac{\ln \text{DNA on filter}}{\text{eluted volume}}$).Each experiment was repeated five times with duplicate DNA determinations and included a treated and a matching control animal.
We found that four hours after administration of the carcinogen there was a substantial and comparable DNA damage in liver and kidney of all strains examined: K higher than 100 over control.The only exception was a comparatively low but still significant value for C57 BL/6N kidney DNA. Conversly, colon DNA damage was hardly above background control in the carcinogen -resistant strains AKR/J :DBA/2 . The highest DNA damage was detectable in the most susceptible strain SWR/J (K >90) while K was about 50 over control for the two other susceptible strains C57 BL/6N ;CD1.Susceptibility to DNA breaks could be one of the factors accounting for genetically determined carcinogen suceptibility.

A26 HETEROGENEITY OF N-MYC EXPRESSION IN CULTURED HUMAN NEURONAL TUMORS. E. Bogenmann. Childrens Hospital of Los Angeles, Los Angeles, CA 90027, USA.

It has long been shown that tumors are composed of heterogeneous cell populations in regard to all growth, invasive and metastatic potentials. We hypothesize that tumors show heterogeneity for gene amplification and expression and we have chosen the N-myc oncogene in human neuronal type neoplasms such as retinoblastoma and neuroblastoma as model system.

Surgically excised tumors were grown in vitro and N-myc amplication and expression is documented by Southern and Northern blot analysis. In situ hybridization of ^{35}S-dATP labeled cDNA to cytosmears or histological sections of cultured tumors demonstrated extensive heterogeneity of N-myc expression. Retinoblastoma and neuroblastoma spontaneously differentiate in the established culture system and we are now analyzing the regulation of N-myc expression during differentiation. In situ localization of N-myc in differentiated cultures will demonstrate correlations of cell differentiation and N-myc expression in neuronal human tumors.

Supported by NIH Grant #EY04950-03.

A27 INCREASED LEVEL OF AMPLIFICATION OF THE C-MYC ONCOGENE IN TUMORS INDUCED IN NUDE MICE BY A HUMAN BREAST CARCINOMA CELL LINE - Olivier Brison[1], Nazanine Modjtahedi[1], Christian Lavialle[2], Marie-France Poupon[2], Rosa-Maria Landin[2], Roland Cassingena[2], and Roger Monier[1]. 1) Institut Gustave Roussy, 39 rue Camille Desmoulins, 94805 Villejuif Cedex. 2) Institut de Recherches Scientifiques sur le Cancer, BP N° 8, 94802 Villejuif Cedex, France. Cell line SW 613-S, derived from a human breast carcinoma, contained double minute chromosomes (DMs) but lost them upon in vitro cultivation. These cells were tumorigenic in nude mice. Cell lines were derived from the tumors and were found to have a high DM content. In three such cell lines, DMs were stably maintained upon in vitro cultivation whereas in another they were lost. The c-myc oncogene is amplified 5- to 10-fold in SW 613-S and 20- to 90-fold in the different cell lines derived from the tumors. The additional c-myc copies were found associated with a purified DM fraction. In cell lines which lost the DMs during in vitro passages the level of amplification was maintained. In situ hybridization experiments indicated that this loss was compensated by the acquisition of copies of the c-myc gene integrated into a chromosome. No major rearrangement of the amplified c-myc was detected. The amount of c-myc mRNAs is roughly proportional to the level of amplification. Our results indicate that growth of SW 613-S cells as tumors in nude mice selected cells with an increased level of amplification and expression of the c-myc oncogene.

A28 EXPRESSION OF THE PUTATIVE MAMMARY ONCOGENE INT-1 CAUSES A MORPHOLOGICAL CHANGE IN MAMMARY EPITHELIAL CELLS. Anthony M.C.Brown, Tom Prendergast, and Harold E.Varmus. Dept. of Microbiology and Immunology, University of California, San Francisco, CA 94143.

The majority of mammary tumors induced by Mouse Mammary Tumor Virus in C3H mice are clonal outgrowths of cells in which an MMTV provirus has inserted adjacent to the int-1 gene. The proviruses appear to activate expression of int-1 RNA, and the gene is strongly implicated in tumorigenesis.

In order to investigate directly the function of int-1 we have constructed MLV-based retrovirus vectors containing the int-1 gene together with a neomycin resistance marker, and used the viruses to infect a mammary epithelial cell line in culture. Ψ2 packaging cells were infected with helper-free MLVint neo virus stocks derived from the amphotropic packaging cell line PA12, and single colonies were selected with G418. Analysis of the proviruses in the Ψ2 cells indicated that the three introns of the int-1 gene had been efficiently removed by splicing. Helper-free virus from these Ψ2 cells was then used to infect the mammary epithelial cell line C57MG, derived from the normal mammary gland of a C57 mouse. The uninfected cells have a regular cuboidal appearance at confluence, while cells infected with the int neo virus were strikingly more elongated and refractile. Cells infected with a control virus, containing a frameshift mutation near the start of the int-1 coding region, appeared similar to the uninfected cells. The morphological change is therefore specific for int-1. Other transformation parameters in these cells are currently under investigation.

A29 A MONOCLONAL ANTIBODY SPECIFIC FOR AN ACTIVATED RAS P21, W.P. Carney, D. Petit, P. Hamer, H. Wolfe, G. Cooper, and H. Rabin, E.I. DuPont deNemours, Biomedical Products Department, No. Billerica, Mass. 01862, Dept. of Pathology, Cancer Tufts New England Medical Center, Boston, MA, and Dept. of Pathology, Dana Farber Cancer Institute, Boston, MA.

Activated ras transforming genes encode proteins with amino acid substitutions at positions 12, 13 and 61 and have been detected in 10-20% of human neoplasms. Since the products of activated ras genes are structurally distinct from their normal homologs we investigated the feasibility of developing MOAbs which react specifically with activated ras proteins at position 12. A MOAb, designated DWP, was raised against a ras-related synthetic dodecapeptide corresponding to amino acids 5-16 of an activated ras p21 containing valine rather than the normal glycine at position 12. In order to biochemically define the specificity of DWP, cellular p21s containing various amino acid substitutions at position 12 were evaluated by the Western blot procedure for reactivity with Y13-259, DWP or MOPC141. Results show that DWP reacts with $p21s$ of NIH cells transformed with DNA from either the activated EJ bladder carcinoma (ras^H) or the colon carcinoma SW480 (ras^K) which both encode the valine mutation at position 12. In contrast, DWP did not react with cells containing normal p21 (glycine at 12), or cells transformed by viral ras^K (serine at 12), or viral ras^H (arginine at 12). In addition, DWP did not react with NIH cells transformed by activated ras genes encoding either cysteine or asparatic acid at position 12. DWP may be a valuable reagent in determining the presence and frequency of mutated ras genes in tumors.

A30 DIFFERENTIAL EFFICIENCIES OF IN VITRO TRANSLATION OF MOUSE C-MYC TRANSCRIPTS DIFFERING IN THE 5'UNTRANSLATED REGION, Andre Darveau, Gobinda Sarkar and Nahum Sonenberg, Department of Biochemistry, McGill University, Montreal, Que. Canada H3G 1Y6. We have studied the in vitro translational efficiencies of two murine transcripts synthesized in vitro that differ in the lengths of their 5' noncoding regions (448 and 83 nucleotides). When translated in a reticulocyte translation system, the shorter transcript translated 10 times more efficiently. The existence in the human c-myc gene of a 75 bp region in exon 1 highly complementary to a region in exon 2 which could form a stable hairpin loop in the mRNA was previously reported (Saito, H. et al. 1983, PNAS USA, 80, 7476-7480). Computer analysis of potential mRNA secondary structure shows that this putative hairpin loop structure could also occur in the mouse c-myc mRNA. However, c-myc mRNA with long 5' non coding regions but lacking the 75 nt which are involved in the formation of the hairpin loop did not show increased translational efficiency. The insertion of these nucleotides in front of the short c-myc transcript reduced only slightly the translational level of the message. To see if the 5' noncoding region could affect the translational level by itself without interacting with any other part of the c-myc message, we juxtaposed a major portion of this region in front of other mRNAs. The in vitro translational efficiencies of these messages were greatly reduced by the presence of this region at their 5' end. The 5' noncoding region of the normal full length mouse c-myc mRNA thus interferes with efficient translation. Rearranged c-myc mRNA generated in some hematopoietic malignancies in which 5' non coding sequences from exon 1 are lost might therefore be translated in vitro with increased efficiency, thus contributing to neoplastic transformation.

A31 Effect of mutations in the human c-myc gene upon transformation potential, DNA-binding and nuclear localization. Titia de Lange, William Lee, James Stone, Gary Ramsay, J. Michael Bishop and Harold E. Varmus. Dept. Microbiol. and Immunol., UCSF, San Francisco, CA94122.

In order to define functional domains within the c-myc protein we have made 12 linker-insertion and 19 deletion mutations in the coding region of the human c-myc gene. We have assessed the activity of these genes in the rat embryo cell co-transformation assay, using EJras as the cooperating oncogene. Our results indicate that at any of 7 positions within the first two-thirds of the 439 amino acid protein can be disrupted by insertion of a few amino acids without loss of activity. In addition, deletion of the central 25% or of a short sequence near the amino-terminus does not affect the transformation potential. In contrast, sequences around amino acid 104 and near the carboxy-terminus are essential for transformation. The same mutated alleles are being tested for two additional characteristics of the c-myc protein: nuclear localization and DNA-binding. The results of these analyses will be presented.

A32 THE *met* ONCOGENE EXPRESSES A TRUNCATED mRNA WITH HOMOLOGY TO THE TYROSINE PROTEIN KINASES.

Michael Dean[1], Morag Park[2], Terry S. Robins[1], Donald G. Blair[2], George F. Vande Woude[1]
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The *met* transforming gene was isolated from a chemically-treated human osteosarcoma cell line (MNNG-HOS) using the NIH3T3 cell transfection assay. *met* is unrelated to other known oncogenes or transforming genes and has been mapped to the long arm of human chromosome 7. Two classes of *met*-related transcripts are differentially expressed in human cell lines indicating a cell type or tissue-specific mechanism of gene control. We also show that the MNNG-HOS cell line expresses a new *met* RNA transcript of 6.5 kb when compared to the nontumorigenic HOS cell line. NIH3T3 *met* transformants express only this aberrant RNA which is shorter than either of the *met* Class I (11 kb) or Class II (12 kb) RNAs detected in human cells. Mapping data suggest that the 6.5 kb RNA has the correct 3' Class I RNA end but has an altered 5' end when compared with the HOS cell RNAs. The *met* transforming activity therefore is associated with the expression of a truncated Class I RNA product. Partial nucelotide sequencing data reveals that the Class I portion of *met* is homologous to the tyrosine protein kinase oncogenes and growth factor receptors. We postulate that the *met* oncogene expresses a truncated kinase domain of the larger *met* proto-oncogene product.

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A33 THE REGULATED EXPRESSION OF THE C-MYC AND C-MYB ONCOGENES DURING ERYTHROID DIFFERENTIATION. Ethan Dmitrovsky, Shoshana Segal, Gregory Hollis, and Ilan Kirsch. NCI/Navy Medical Oncology Branch, Naval Hospital, Bethesda MD 20814. Cellular differentiation is a complex process and the mechanisms involved are at present poorly understood. We investigated the expression of the genes c-myc, c-myb, and alpha globin in two mouse erythroleukemia cell lines which can be induced to terminally differentiate when exposed to dimethylsulfoxide. We found a reciprocal relationship between the cells production of messenger RNA for these oncogenes and globin as well as an early transient disappearance of the oncogene messages prior to evidence of differentiation. We have considered a cause-effect interaction between the diminished oncogene message and cellular progression to differentiation. To test this possibility, we have transfected the human c-myc oncogene into two mouse erythroleukemia cell lines. We are investigating the role of this transfected gene in the differentiation of these cells.

A34 SPECIFIC GROWTH RESPONSE OF ras TRANSFORMED EMBRYO FIBROBLASTS TO TUMOR PROMOTERS

G. Paolo Dotto, Luis F. Parada, and Robert A. Weinberg, Whitehead Institute Cambridge, Massachusetts 02142

Chemical carcinogenesis is a process involving multiple steps, as shown in several in vivo experimental systems. Two early steps have been well characterized: initiation, achieved by a single, subthreshold dose of a carcinogen, and promotion, induced by repetitive treatments with a non-carcinogenic tumor promoter. At the cellular level, establishment of the transformed phenotype is also a multistep process and activation of several, independent genes appears to be required. Here we show that, similarly to initiated cells, primary rat embryo fibroblasts (REFs) containing a ras but not a myc oncogene are strongly and specifically stimulated to grow by tumor promoters. In the presence of these substances, ras-containing REFs acquire the ability to over-grow normal cells in the monolayer and form foci with 100% efficiency. Similar to the in vivo situation, promoter effects can be blocked by the concomitant application of retinoic acid.

A35 LOCALIZATION OF THE CHROMOSOME 21 HU-ETS-2 GENE AND TRANSLOCATION IN t(8;21) AML, Harry Drabkin[1,2], Margaret Van Keuren[1], N. Sacchi[3], Takis Papas[3], and David Patterson[1,4]. [1]Eleanor Roosevelt Institute for Cancer Research, [2]Dept. of Oncology, [4]Depts. of Biochem., Biophys., Chemistry and Dept. of Medicine, Univ. of Colo. Health Sciences Ctr., Denver CO 80262; [3]Fredericks Cancer Facility, Frederick MD 21701

We have used somatic cell hybrids to precisely localize Hu-ets-2 DNA sequences on chromosome 21. These hybrids contain separately both derivative chromosomes from the AML 8;21 translocation, t(8;21)q22.1;q22.3), and a ring chromosome 21 with a breakpoint also in 21q22.3. Hu-ets-2 DNA sequences are located in band q22.3 within the small overlap region between the t(8;21) and ring chromosome 21 breakpoints. Hu-ets-2 is proximal to the DNA sequence pPW231C which is at the breakpoint in the ring chromosome 21. Hu-ets-2 is translocated in t(8;21) AML confirming the reciprocal nature of this translocation. This is ERICR #600. This work was supported by HD17449, AG00029, the Milheim Foundation for Cancer Research, and an NCI Clinical Investigator Award (H.D.).

A36 THE VIRAL p21 RAS PROTEIN IS A POTENT MITOGEN WHICH STIMULATES ADENYLATE CYCLASE ACTIVITY IN THE EARLY G_1 PHASE OF CULTURED RAT CELLS, Jon P. Durkin, D.J. Franks and J.F. Whitfield, Division of Biological Sciences, National Research Council of Canada, Bldg. M-54, Montreal Road Campus, Ottawa, Canada K1A 0R6

tsKNRK rat cells infected with a temperature-sensitive Kirsten-sarcoma virus mutant were arrested in the G_0/G_1 state by incubation in serum-free medium at a temperature (41°C) which inactivates the virus' abnormally thermolabile 21 kD RAS protein product (p21). Lowering the incubation temperature to 36°C reactivates p21 which rapidly induces G_1 transit and DNA synthesis (8-10 hours later) in these serum-deprived cells. Reactivating the viral RAS protein also caused a rapid (within 1 hr) and transient increase in cellular adenylate cyclase activity. The 41°C to 36°C shift did not affect the enzyme's activity or stimulate G_1 transit in serum-deprived, uninfected NRK cells. RAS activation sensitized adenylate cyclase to GTP and to stimulation by forskolin. Thus, we demonstrate that the oncogenic/mitogenic viral RAS protein is able to stimulate adenylate cyclase and G_1 transit in a mammalian cell line just as other RAS proteins have been shown to do in yeast.

A37 IDENTIFYING RAS AMINO ACIDS INVOLVED IN GUANINE NUCLEOTIDE BINDING, Larry A. Feig, Bin Tao Pan, Tom Roberts and Geoffrey Cooper, Dana Farber Cancer Institue Boston, Mass 02115

A method has been developed to isolate mutant ras proteins that are defective in guanine nucleotide binding activity. It involves randomly mutagenizing a v-ras^{H} bacterial expression vector then selecting for bacteria harboring mutant proteins by an in situ GTP binding assay on lysed bacterial colonies. The first two mutants analyzed revealed that ras amino acids 119 and 83 influence nucleotide binding activity since amino acid substitutions at these sites decreased the Kd of p21 for GTP and GDP by 100 and 30-fold respectively. At codon 119,aspartic acid was replaced by asparagine while at codon 83 alanine was replaced by threonine. In DNA transfection assays, both mutant genes gave rise to foci of transformed NIH 3T3 cells with efficiencies equal to that of a wild-type v-ras^{H} gene. Apparantly, the mutant proteins still bound GTP in vivo since in NIH transformants the mutant p21s were autophosphorylated to the same degee as wild-type v-ras^{H} p21.

A38 EXPRESSION AND ACTIVITY OF EGF RECEPTOR IN HEAD AND NECK SQUAMOUS CARDINOMA CELL LINES. G.E. Gallick, P.G. Sacks, S.A. Maxwell, P.A. Steck, and J.U. Gutterman, University of Texas M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Epidermal growth factor receptor (EGF-R) is frequently overexpressed in squamous cell carcinomas, although the significance of this expression to tumorigenicity remains unknown. We have been examining EGR-R in two recently established squamous cell carcinomas of the head and neck. In cell line 183A, expression and activity of EGF receptor is equivalent to that seen in normal eipthelial cells. In contrast, 1483 cells are elevated 8-10 fold in rate of synthesis of EGF-R, as determined by metabolic labeling studies, and 4-6 fold in steady state level of the receptor, as determined by immunoblotting and ^{125}I-EGF binding studies. 1483 cells also have an increased rate of EGF-R degradation. Both cell lines produce an enzymatically active EGF-R capable of autophosphorylation in immune complex kinase assays. However response of enzymatic activity to EGF stimulation is decreased in 1483 cells, with only a 2 fold stimulation of activity observed, compared with a 6 fold stimulation observed in 183A cells. Possible explanations for these results include that EGF receptors of 1483 cells may be "activated" independently of EGF binding, either because an altered form of the receptor is synthesized, or because of cooperativity between EGF receptors on the cell surface. In addition to EGF receptors, levels of p21^{ras} were also increased approximately 20 fold in 1483 cells with respect to 183A cells. These results suggest that changes in EGF receptor expression might be associated with altered c-ras gene expression, a possibility under investigation.

A39 Viral Oncogene Activation of Macrophage Differentiation in vitro. CE Gee,V Cherington,L Sastre, FJ King, TS Springer and TM Roberts.

Phorbol ester(TPA) has been shown to induce differentiation of myeloid cells. This induction is presumed to be mediated by protein kinase C, which is also activated physiologically by diacylglycerol (DAG). The middle T antigen of polyoma virus is involved in transformation and tumorigenesis in animals and in culture. The in vitro transforming activity is strongly correlated with its ability to induce phosphorylation of phosphotidylinositides, increasing inositol triphosphate and DAG second messenger levels in cells. We have evidence that the polyoma middle T antigen induces cellular differentiation of murine myelomonocytic cells into macrophages in culture, thus mimicking the effect of TPA. Middle T cDNA was inserted into a retroviral vector (pZIPNeoSV(X)1), containing the coding region for neomycin resistance. This construct was introduced into a myelomonocytic cell line, Ml. The G418-resistant middle T-infected cells were shown to produce the middle T protein by immunoprecipitation. By immunofluorescence flow cytometry analysis, the middle T-infected cells were shown to express a differentiation marker, the receptor for complement C3bi (Mac-1), on the cell surface. The amount of Mac-1 present on these cells is comparable to that of the Ml cells induced to differentiate with other stimuli. Cells infected with a retroviral vector by itself or a vector containing polyoma large or small T antigen cDNA, or a vector containing SV40 large T antigen were negative for activation of Mac-1 expression or expression of any other differentiation characteristic. We are investigating the mechanism of activation of differentiation induce by the viral oncogene, polyoma middle T antigen.

A40 N-myc EXPRESSION IN NORMAL FETAL RETINA AND RETINOBLASTOMA: NEW CLUES ABOUT THE TARGET CELL FOR RETINOBLASTOMA TUMORIGENESIS, Audrey Goddard, Jeremy Squire, Marc Canton, Brenda Gallie, Robert Phillips, Dept. of Medical Biophysics, University of Toronto, and the Ontario Cancer Institute, Toronto, Canada M4X 1K9.

The childhood eye cancer retinoblastoma (RB) can be transmitted as an autosomal dominant gene. RB tumors probably arise from an embryonic retinal precursor cell. Recently there have been reports of gene amplification and expression of the oncogene N-myc in two of the childhood neuroectodermal tumors, RB and neuroblastoma. In our studies of RB, we found that N-myc gene amplification was rare (1 of 16 previously unpublished tumors), however Northern blotting demonstrated the N-myc RNA was present in all tumors tested (6/6) and was also expressed at similar levels in human fetal retinas of 6-12 week gestational age. Densitometric measurements indicated that the tumors with diploid N-myc genes (4/6) still had high levels of N-myc RNA (10-30%) when compared to tumors with N-myc amplification (2/6). We conclude that the expression of N-myc in RB is not a late activation event conferring increased tumorigenicity but probably results from the normal cellular expression levels present in target retinal progenitors. Recent evidence suggests that RB is a recessive gene at the cellular level; one possible function of the normal product of the RB gene may be the suppression of oncogenes during retinal differentiation. Specific genomic amplification of N-myc implies that N-myc may have functional importance to some tumors, however the low frequency we have observed suggests that this mechanism is not as significant in RB as it is in neuroblastoma.

A41 MOLECULAR CLONING OF THE NEU GENE: ABSENCE OF GROSS STRUCTURAL CHANGE IN ONCOGENIC ALLELES, Mien-Chie Hung, Pierre-Yves M. Chevray, Cornelia I. Bargmann, Alan L. Schechter, David F. Stern and Robert A. Weinberg
Whitehead Institute for Biomedical Research, Cambridge, MA 02142

The neu gene is distantly related to the erbB gene and encodes a cell surface protein that appears to function as a growth factor recptor. In order to study the mechanisms that caused the conversion of the normal neu gene to an oncogenic alleles, we have isolated molecular clones of the neu oncogene as well as a clone of the corresponding protooncogene. The transforming neu oncogene and the proto-neu gene clones exhibit identical restriction enzyme patterns. Amplification of the proto neu gene in NIH3T3 cells via cotransfection with a dihydrofolate reductase gene resulted in methotrexate-resistant colonies that produce high levels of normal neu-encoded, p185 protein. In contrast to cells carrying low levels of the oncogene-encoded protein, these cells appeared normal. The results suggest that the lesion that led to activation of the neu gene is a minor change in DNA sequence and is apparently located in the protein-encoding region of the gene. We are now in the process of determining the molecular alteration(s) that distinguish the proto-neu and oncogenic forms of neu.

A42 CHARACTERIZATIONS OF ANTIGENS RECOGNIZED BY HUMAN MONOCLONAL ANTIBODIES TO MAMMARY CARCINOMA CELLS, Ashraf Imam and Clive R. Taylor, University of Southern California, Los Angeles, CA 90033

The aim of this study was to generate and identify human monoclonal antibodies to autologous mammary carcinoma cells by the host's immune response. Hence, the lymphocytes from lymph nodes of 3 patients with metastatic mammary carcinomas were hybridized by fusing them with a non-secreting variant of murine myeloma cells. A total of 19 immunoglobulin G and 5 immunoglobulin M human monoclonal antibodies which showed strong reactivity to mammary carcinoma cells in tissue sections by an indirect immunoperoxidase staining method were obtained. Two immunoglobulin G monoclonal antibodies (designated AW-5G and RR-2G) were selected, on the basis of their strong reactivity to the tumor cells, to identify their corresponding antigens and to study their patterns of binding and specificity to cells in formalin-fixed and paraffin-embedded tissue sections of a) primary and regional metastatic infiltrating and lobular breast carcinomas, b) benign breast disease and c) normal breast. Under these conditions, the malignant mammary epithelial cells were discriminated from their normal counterparts. The antibodies reacted with variable intensity to cytoplasmic components of the malignant cells and only weakly with benign breast disease. The lymphocytes, monocytes and erythrocytes were unreactive. The antibodies AW-5G and RR-2G immunoprecipitated a phosphoprotein component (M_r = 29,000), and two protein components (M_r = 31,000 and 34,000) respectively from lysates of intrinsically labelled human mammary carcinoma cell line (MCF7). Thus, these antibodies appear to be useful to identify and characterize both structurally and functionally their corresponding antigens which are present in elevated levels in malignant cells of mammary gland. The mechanism by which these components may regulate growth and/or differentiation of tumor cells is being studied.

A43 FUNCTIONAL ACTIVATION OF MURINE PERITONEAL MACROPHAGES WITH BACTERIAL LIPOPOLYSACCHARIDE ALTERS EXPRESSION OF C-FOS AND C-MYC ONCOGENES

M. Introna, T. A. Hamilton, R. E. Kaufman, G. Strassman, D. O. Adams, R. C. Bast; Duke University Medical Center, Durham, NC

Evidence has accumulated that c-fos and c-myc transcription increases transiently following stimuli which induce proliferation of nonmalignant cells. Expression of the same oncogenes also varies during differentiation of immature progenitors to functionally mature cells. We have studied the behavior of c-fos and c-myc in a different setting, i.e., the functional activation of fully differentiated, non-proliferating murine peritoneal macrophages. Triggering of functional activation in these cells following expression to lipopolysaccharide (LPS), alters the expression of the c-fos and c-myc oncogenes. After exposure to LPS (20 ng/ml), at least four phases could be distinguished: 1) a prompt disappearance of RNA messages for c-fos and c-myc, 2) a peak of newly transcribed mRNA for both genes, 3) a subsequent disappearance of the signal, and, 4) a re-accumulation of mRNA. A similar pattern of c-fos and c-myc expression could be induced with PMA, suggesting that some of the effects of LPS could be mediated through protein kinase C. Macrophages did not proliferate when exposed to LPS and changes in c-fos and c-myc RNA could not be attributed to cell-cycle related activation, but rather with the acquisition of the ability to perform specialized functions.

A44 THE HUMAN N-*myc* GENE IS CLOSELY RELATED IN ORGANIZATION AND NUCLEOTIDE SEQUENCE TO THE c-*myc* GENE

Nancy E. Kohl, Edith Legouy, Connie E. Gee, Ronald A. DePinho, Perry Nisen, Russell Smith and Frederick W. Alt, Columbia University, New York, NY, 10032.

We have determined the structure of the human N-*myc* gene and the nucleotide sequence of its mRNA product. N-*myc*, like c-*myc*, is organized into three exons. Both genes share the remarkable property of having a large, untranslated leader sequence encoded primarily by the 5' exon, although no homology is detected between the non-coding exons of the two genes. Translation of the putative N-*myc* protein initiates near the 5' end of the second exon and continues for 464 amino acids. Comparison of the deduced amino acid sequence of this protein with that of the human and viral *myc* proteins indicates that the three proteins share regions of significant homology in the domains encoded by both exons 2 and 3. These data provide evidence for the classification of N-*myc* as a member of the *myc* family of proto-oncogenes.

A45 PROTEIN-TYROSINE KINASE ACTIVITIES OF TRUNCATED EGF RECEPTOR PROTEINS PRODUCED BY ACUTE OR CHRONIC RETROVIRAL INFECTION

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Subversion of the mitogenic signal through expression of a truncated EGF receptor seems to be involved in transformation by the oncogenic protein, v-erB, carried by the acutely transforming avian erythroblastosis virus (AEV). This protein possess extensive homology with the protein-tyrosine kinase domain of both the human EGF receptor as well as members of the protein-tyrosine kinase family of oncogenes. Indeed, the v-erB protein contains measurable protein-tyrosine kinase activity. Chicken erythroblastosis is thought also to be mediated by promotor-insertion of the chronically transforming avian leukosis virus (ALV) into the c-erB (EGF receptor) gene. The v-erB related proteins that are produced by the ALV leukemias were analyzed and compared to the EGF receptor and v-erB protein. The two leukemias analyzed produced truncated proteins of 78,000 MW and 88,000 MW which are immunoprecipitated by antiserum which recognize the c-terminal domain of the EGF receptor. These truncated proteins become phosphorylated upon the addition of ATP (gamma 32-P). Thus, it seems likely that acute and chronic retroviruses utilize a common pathway for transformation. The proteins expressed in the leukemias are similar to the avian EGF-receptor with respect to their phosphopeptide map thus apparently lacking the c-terminal deletion characteristic of V-erB, thus suggesting that this deletion is not required for the induction of erythroblastosis.

A46 ENHANCED EXPRESSION OF c-myc ONCOGENE IN PAPILLOMAVIRUS ASSOCIATED CARCINOMAS OF THE UTERINE CERVIX AND ITS PRONOSTIC SIGNIFICANCE. Guy Riou, Michel Barrois, Martine George* , Jean-Pierre Wolff* and Gérard Orth**. Laboratoire de Pharmacologie Clinique et Moléculaire. *Service de Gynécologie. Institut Gustave Roussy, 94800 Villejuif et **Unité des Papillomavirus (INSERM U 190), Institut Pasteur, 75015 - Paris (France).

The expression of the c-myc oncogene was studied in 41 specimens of invasive squamous cell carcinomas of the uterine cervix at different clinical stages. HPV DNA sequences were detected in 90 % of the tumor samples. Total RNA was analyzed by the Northern blot hybridization technique and the c-myc transcripts were revealed by hybridization with the third exon (Eco RI - Cla I DNA fragment) of the human c-myc oncogene. The presence of a 2.4 kb c-myc RNA was shown in all RNA samples analyzed, and with a weak intensity in the RNA extracted from normal human tissues (cervix, thyroid and lung). The level of c-myc expression was evaluated by dot blot analysis. A 4-21 fold enhanced transcription of c-myc gene was observed in 3/9 stage 1, 6/11 stage 2, 10/12 stage 3, 3/3 stage 4 and 5/5 recurrent tumors and in one lymph node metastatic tumor. These results strongly suggest that the activation of the c-myc oncogene is associated with tumor progression. Furthermore, a rapid evolution towards more advanced disease was observed in 6 out of the 9 tumors of stage 1 or 2 with high levels of c-myc RNA. This suggests that the overexpression of the c-myc gene is a factor of clinical pronostic significance for carcinomas of the uterine cervix at stages 1 and 2.

A47 CIS COACTIVATION OF QUIESCENT METALLOTHIONEIN GENES BY CARCINOGENS IS ASSOCIATED WITH DEMETHYLATION OF SEQUENCES UPSTREAM OF THE GENES. Craig A. MacArthur, Teresa Hawley, and Michael W. Lieberman. Fox Chase Cancer Center, Philadelphia, PA 19111.

We have studied the activation of transcriptionally quiescent metallothionein (MT) genes in cadmium-sensitive S49 mouse lymphoma cells by carcinogens. Treatment of MT^- S49 cells with ultraviolet radiation, N-ethylnitrosourea, or N-acetoxy-2-acetylaminofluorene results in up to a 20-fold increase in phenotypically stable cadmium-resistant (Cd^r) variants. Steady state RNA analysis reveals that over 70% of the Cd^r variants make MT RNA, and of the MT^+ variants, nearly 50% make both MT-I and MT-II RNA. Nuclear transcription studies show that the MT genes are transcriptionally co-activated in $MT\text{-}I^+/MT\text{-}II^+$ variants and that the coactivated MT genes are coordinately regulated by cadmium at the transcriptional level. Using a transfection assay, we have shown that MT^- S49 cells contain any trans factor(s) necessary for basal and cadmium-regulated expression of a gene linked to a MT-I promoter and other upstream sequences. This suggests that carcinogen activation of the linked MT genes in S49 cells occurs by mechanisms which ultimately act in cis. Demethylation at Hpa II sites upstream of the MT genes is correlated with activation, supporting the concept of cis activation. We detected no insertions, deletions, amplifications, or rearrangements of the MT locus. Hence carcinogens transcriptionally coactivate quiescent MT genes in MT^- S49 cells by mechanisms which act in cis and result in demethylation upstream of the expressed genes. (Supported by NIH Grant CA39392).

A48 ALTERATION OF c-fes EXPRESSION DURING INDUCED DIFFERENTIATION OF HUMAN LEUKEMIC CELLS. Ian MacDonald, Julia Levy, and Tony Pawson*. Department of Microbiology, University of British Columbia, Vancouver, B.C., Canada and *Department of Medical Genetics, University of Toronto, Toronto, Ontario, Canada.

The retroviral fes(fps) oncogene encodes a transforming protein with an associated tyrosine kinase activity. By employing a cross-reactive rat anti-$P140^{gag\text{-}fps}$ serum we immunoprecipitated and characterized a 92Kd protein from normal and leukemic human cells. This protein was designated $p92^{c\text{-}fes}$ on the basis of similarity of M.W. with known fes/fps proteins, tyrosine kinase activity, and comparative proteolytic mapping data with known viral and cellular fes proteins. The expression of $p92^{c\text{-}fes}$ was found in humans to be limited to hematopoietic cells and cell lines, and this protein could be detected in both normal and leukemic leukocytes. In addition to $p92^{c\text{-}fes}$ we also identified a 94Kd protein with antigenic cross-reactivity to $p92^{c\text{-}fes}$ and possessing tyrosine kinase activity. However, this 94Kd protein was not hematopoietic specific since it was expressed in many non-hematopoietic cells. The tumour promoter TPA(tetradecanoyl-phorbol-myristic acetate) causes some human leukemic cells to differentiate, and the effect of this agent upon c-fes expression has been examined and the data will be presented. Also, our observations of c-fes expression in TPA-responsive and TPA-unresponsive cell lines will be reviewed.

A49 ARE ras PROTEINS INVOLVED IN POLYPHOSOINOSITIDE BREAKDOWN? Veeraswamy Manne and Hsiang-fu Kung, Hoffmann-La Roche Inc., Department of Molecular Oncology, Nutley, NJ 07110.

Polyphosphoinositides play an important role in signal transmission for a major group of hormones, neurotransmitters and growth factors. The earliest response detected upon the interaction of the ligands with their receptors was the breakdown of phosphatidylinositol 4,5 biophosphate into inositol triphosphate and diacylglycerol, catalyzed by phospholipase C. Both inositol triphosphate and diacylglycerol have been proposed to act as second messengers. The nature of proteins involved in the coupling of receptors to phospholipase C is unknown. However, several lines of indirect evidence strongly suggest that a GTP-binding protein is involved. The Ha-ras gene product, p21, which is involved in the control of cellular growth is a membrane protein that binds guanyl nucleotides and hydrolyzes GTP. Based on the analogy with other guanyl nucleotide binding proteins, several groups have proposed that p21 might be involved in the signal transduction process across the membrane. H-ras p21 proteins synthesized in E. coli have been highly purified. Attempts have been made to test the possibility that p21 is involved in the coupling of receptors to phospholipase C. We have identified two phospholipase C enzymes in the cytosolic fraction of platelets. These two enzymes differ in their optimum pH, sensitivity to guanidine hydrochloride and their requirement for Ca^{2+}. One of the enzymes seems to be specific to phosphatidylinositol whereas the other acts on phosphatidylinositol 4,5-biphosphate preferentially. We are in the process of purifying these enzymes to homogeniety and will be testing the effect of normal as well as activated ras proteins on the enzymatic activities.

A50 PKS, a New RAF Related Sequence in Man: G. Mark[1], T. Shows[2] and T. Seeley[2], [1]National Cancer Institute, Bethesda, MD; [2]Roswell Park Memorial Institute, Buffalo, NY.

A human fetal liver library, screened at reduced stringency for v-raf related sequences, revealed inserts representing the c-raf-1 locus and a novel cDNA. Appropriate regions of the new gene (pks, for protein kinase sequence) were sequenced and compared to the other raf homologs. pks exhibits a 70.5% and 72% nucleotide homology to c-raf-1 and v-raf, respectively. Comparison of the protein sequences reveals: a homology of 75% to the

A51 A MODEL FOR THE TERTIARY STRUCTURE OF p21, THE PRODUCT OF THE RAS ONCOGENE, Frank McCormick[1], Brian F.C. Clark[2], Trols F.M. La Cour[2], Morten Kjeldgaard[2], Leif Norskov-Lauritsen[2], and Jens Nyborg[2], [1]Dept. of Human Genetics, Cetus Corporation, Emeryville, CA 94608 and [2]Aarhus University, Aarhus, Denmark.

A model has been generated for the tertiary structure of p21, the product of the ras oncogene. The model is based on the tertiary structure of the GTP/GDP binding domain of bacterial elongation factor EF-Tu, with which p21 shares sequence homology: 42% of p21 amino acids can be aligned with identical or conservative substitutions in EF-Tu. The model predicts that p21 consists of a central core of beta sheet structure, connected by loops and alpha helices. The guanine nucleotide binding region is made up of four of these loops (amino acids 10-16, 57-63, 115-128 and 144-160). The phosphoryl binding domain contains sequences from 10 to 16. This proposal is supported by immunological and genetic data relating to the role of position 12 in GTP binding and hydrolysis. Sequences from 57-63 are close to the phosphoryl binding region and may contain a site for magnesium binding. Amino acids defining guanine specifically are asparagine-116 and aspartate-119, and sequences around 145 may contribute to guanine binding.

We have made mutants of human N-ras, expressed in E. coli, to test various aspects of this model. For example, mutations at 116 or 119 eliminate GTP binding. The properties of other mutations in or around the proposed GTP binding site will be presented. Using this model, we have been able to predict which regions of p21 are likely to interact directly with cellular components, and have been able to test these predictions with anti-peptide antibodies against these regions of the proteins.

A52 TRANSFORMATION OF NORMAL HUMAN FIBROBLASTS WITH V-SIS VIA DNA TRANSFECTION, J. Justin McCormick, Dennis G. Fry, and Veronica M. Maher, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824

The oncogene (v-sis) of simian sarcoma virus (SSV) has a high degree of homology to the cellular gene for platelet-derived growth factor, a potent mitogen for fibroblasts in culture. The cellular homolog of v-sis has been found to be activated in some human tumors and tumor cell lines. We constructed plasmids in which the SSV-provirus is linked to a dominant selectable marker for mammalian cells, i.e., resistance to either Geneticin or mycophenolic acid. We used a DNA transfection technique to insert this recombinant plasmid into normal human fibroblasts. Following selection of antibiotic resistant cells, we detected the presence of the transfected v-sis gene by hybridization analysis within cellular genomic DNA and 4-8 times background levels of sis RNA. The cells transfected with the recombinant plasmid formed foci, grew to higher densities, and had altered growth kinetics when compared to cells transfected with the vector plasmid alone. When transfected cells were allowed to grow in the original dishes without antibiotic selection, foci formed. The sis transformed cells had a finite lifespan in culture and were not tumorigenic in athymic mice. Supported by DOE Contract 0645.

A53 Transformation of Human Mammary Epithelial Cells with Oncogenic Retroviruses, R. Milley[1], E. O'Rouke[1], M. Trahey[1], M. Stampfer[2], M. Kriegler[3], F. McCormick[1] and R. Clark[1]. [1]Cetus Corportation, 1400 Fifty-Third Street, Emeryville, CA 94608, [2]Lawrence Berkeley Laboratories, Building 934, Berkeley, CA 94720, and [3]Fox-Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, PA 19111.

We have been developing an assay for mammary carcinoma oncogenes. A cell line (184-A1) derived by benzy(A)pyrene treatment of cultures normal human breast cells was used as an oncogene recipient. To determine selective criteria for oncogenically transformed 184-A1 cells, known oncogenes (ras, mos and SV40 T antigen) were introduced via murine retroviruses. Each of these oncogenes enabled the 184-A1 cells to grow in a selective medium. This result demonstrates the potential utility of these cells for oncogene detection and isolation. Acquisition of anchorage independence by the viral transformants will also be discussed. 184-A1 cells transformed by v-Ha-ras were found to be weakly tumorigenic in nude mice while 184-A1 cells transformed by SV40 T-antigen were non-tumorigenic. Transformants containing both oncogenes, however, were strongly tumorigenic. Similarly, v-mos enhanced the tumorigenicity of ras transformants, especially by increasing their metastatic potential. These results demonstrate the stepwise acquisition of the fully malignant phenotype by primary human mammary epithelial cells in vitro.

A54 SPECIFIC INTERACTION BETWEEN p53 AND MAJOR HEAT SHOCK PROTEINS.
Moshe Oren, Dan Michalovitz, Orit Pinhasi-Kimhi and Avri Ben-Zeev, The Weizmann Institute of Science, Rehovot 76100, Israel.

Transformation of cells by SV40 is accompanied by a marked increase in cellular p53 levels. This increase is due to the stabilization of p53, probably via the formation of a specific complex between p53 and the SV40 T antigen. In non SV40-transformed overproducers of p53 there is also a concomitant stabilization of this protein. We now demonstrate that in such cells p53 is complexed with another cellular protein of ca. 70 kd, which may account for its stabilization. Furthermore, we show that the latter protein is in fact the semi-inducible heat shock protein HSP70, and that p53 following exposure to heat-shock can complex also with the inducible HSP68. Finally, at least in some p53 overproducers, there is also a substantial increase in cellular HSP70 levels. These findings suggest an analogy between HSP70 and the SV40 T antigen, which is also supported by some common features of these two proteins.

A55 ONCOGENESIS OF PRIMARY HUMAN CELLS BY A COMBINATION OF BK VIRUS DNA AND THE HUMAN Ha-ras ONCOGENE. Mary M. Pater and Alan Pater, Faculty of Medicine, Memorial University of Newfoundland, St. John's, NF A1B 3V6 Canada

Primary human embryonic kidney (HEK) cells are cultivated routinely under laboratory conditions. However, these cells can be propagated in culture for only three to four passages, after which the cells stop growing and die. Therefore, these cells are ideal for the studies of molecular mechanism of oncogenes by various agents. We have initiated studies of transformation of these cells by the human paporvavirus BK and human Ha-ras oncogene. We have used BKV DNA (cloned at its unique EcoRI sites in the late region to avoid the lytic infection of HEK cells) for transformation by the focus assay. The cells were transfected with BK-pML plasmid by the calcium phosphate method. Individual foci were selected after 3-4 weeks and the status of viral DNA in each foci was examined. All the foci contain the intact early region of BKV DNA. Also, all the foci express the viral tumor antigens. However, none of the foci are capable of growth in soft agar. We then transfected HEK cells with a combination of BKV DNA and either the activated or the normal form of human Ha-ras. Only HEK cells transfected with a combination of BKV DNA and the activated form of Ha-ras were capable of growth in soft agar. The status of BKV and Ha-ras DNA was examined in selected colonies. All the colonies contain BKV and Ha-ras DNAs in an integrated form. Also all the colonies express the viral tumor antigens and the activated form of Ha-ras. The results thus demonstrate oncogenesis of primary human cells in a multi-step process requiring the expression of more than one oncogene.

A56 STRUCTURAL AND FUNCTIONAL STUDIES ON THE MECHANISMS OF c-myc ACTIVATION IN BURKITT LYMPHOMAS.

Pier-Giuseppe Pelicci, Luisa Lanfrancone, Ethel Cesarman, Daniel M. Knowles, Ian T. Magrath* and Riccardo Dalla-Favera. Dept. of Pathology,Kaplan Cancer Center, New York University School of Medicine, New York, NY, 00006; *Pediatric Oncology Branch, National Cancer Institute, Bethesda, MD, 20205. Structural analysis of translocated c-myc gene in a few cases of Burkitt lymphoma (BL) showed that different types of structural alterations, i.e. truncations and somatic mutations can occur in this locus. In order to gain comprehensive information on the type and location of these alterations, we have mapped them in 36 BL cases by Southern blot hybridization. Our results indicate that : 1)In BL cases displaying c-myc truncations,the breakpoints cluster in an area containing the first exon, its 5' flanking region and the first intron; 2) in BL cases where the c-myc locus is intact, yet mutations of the translocated gene are detectable in the same region as restriction enzyme polymorphisms. Moreover, we observed a strict correlation between the type of structural alteration and epidemiological type of BL, namely mutations within an intact c-myc locus have been found in endemic (African type) cases of BL, while sporadic (American type) cases of BL dispayed c-myc truncations. The detection of structural alterations of the 5' region of the c-myc gene in all BL studied prompted us to examine the role of these sequenses in c-myc regulation. We have constructed recombinant plasmids in which the c-myc first exon and different portions of 5' flanking sequences drive the expression of the bacterial gene coding for chloramphenicol acetyl transferase (CAT). CAT activity has been tested in a transient expression assay upon transfection in NIH/3T3 and HeLa cells. In these cells, relatively low levels of CAT expression were observed with myc vectors containing a 250 bp fragment in front of the first exon. 5' or internal deletions of this fragment led to a 5 to a 10 fold increase in CAT expression. This finding suggests the presence of a negative regulatory element in the 5' flanking region of the c-myc gene. Given this location, it is likely that the regulation occurs at the trascriptional level, as suggested by preliminary run-off trascription experiments. Alterations of this region by truncations or mutations may involved in the activation of the translocated c-myc gene.

A57 THE ACTIVATED HUMAN Ha-ras ONCOGENE CAN BE SILENCED BY IN VITRO METHYLATION.

Marco A. Pierotti, Maria G. Borrello, Italia Bongarzone, Rosangela Donghi, Piera Mondellini and Giuseppe Della Porta. Div. of Exp. Oncology A, Istituto Nazionale Tumori, Via Venezian 1 - 20133 Milano.

We investigated whether the transforming version of the human Ha-ras oncogene, activated by a G→T transversion at the 12th codon, belongs to the class of mammalian genes whose activity is controlled by methylation. The pT24-C3 plasmid was methylated in vitro by MHpa II and MHha I. Three differently methylated plasmids were prepared, two treated with the single enzymes and the third methylated by the two enzymes together. The effectiveness of the reaction was monitored analysing the Southern blotting patterns obtained by digesting the treated and untreated plasmids with the two relevant restriction enzymes. In transfection experiments on NIH/3T3 cells, we obtained a significant reduction (about 80%) of the transforming activity only when the oncogene was double methylated at both Hpa II and Hha I sites. Treatment with 5'-azacytidine, a maintenance methylation inhibitor, of NIH/3T3 cultures transfected with the double methylated gene resulted in a recovery of transformation foci similar to that obtained with the control mock-methylated oncogene. Our results show that a genetic activation of human Ha-ras oncogene can be controlled by epigenetic mechanisms like the methylation of oncogene specific sequences.

A58 RAS GENE ACTIVATION IN RAT TUMORS INDUCED BY VARIOUS BENZIDINE DERIVED DYES,

Steven H. Reynolds, Robert M. Maronpot, John H. Mennear, *Stuart A. Aaronson and Marshall W. Anderson, The National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 and *The National Cancer Institute, Bethesda, MD 20892

Dimethoxybenzidine (DMO) and dimethylbenzidine (DM) are used to synthesize dyes such as C.I. Direct Blue 15 and C.I. Acid Red 114, respectively. These commercially used dyes are metabolically degraded to DMO or DM in the intestinal tract of rodents and subsequentially DMO and DM are absorbed into the blood stream. Animals were exposed to DMO, DM or the dyes in the drinking water. Tumors obtained from treated animals were examined for the presence of activated oncogenes by the NIH/3T3 DNA transfection assay. Activated oncogenes were detected in 79% (15/19) of the squamous cell carcinomas, basal cell neoplasias, clitoral gland neoplasias, and preputical gland neoplasias tested to date. Southern blot analysis of transfectant DNA showed that the transforming properties of the rat tumor DNA were due to the transfer of an activated cellular homologue of either the H-ras or N-ras oncogene. Radioimmunoprecipitation and SDS-polyacrylamide gel electrophoresis showed that the ras proteins from the transfectants exhibited altered electrophoretic mobilities characteristic of point mutated ras genes. The altered electrophoretic mobilities of the ras proteins indicated that different structural mutations are responsible for activation of the H-ras gene within the same tumor type. These data suggest that activation of cellular ras genes by point mutation is an important step in the induction of tumors, at least in rodents, by this class of environmental chemicals.

A59 PRELIMINARY CHARACTERIZATION OF A NOVEL RAS ONCOGENE FROM A NATURALLY-OCCURRING RAT TUMOR
Stephen A. Schwartz and Charles F. Shuler*; The University of Chicago, Chicago, Ill. 60637; The Ohio State University, Columbus, Ohio, 54321*

We previously observed the malignant transformation of NIH/3T3 cells as well as normal rat embryo fibroblasts following a single in-vitro transfection of genomic DNA from a naturally occurring rat sarcoma. The malignant nature of the rat cells was confirmed by the formation of tumors in 8-12 week old rats injected as newborns with transfected rat embryo cells. Although the efficiency of transfection-transformation of the rat cells was significantly lower than for the 3T3 cells, co-transfection with the neo-resistance (Geneticin, G418) gene permitted us to isolate and propogate rat cells successfully transformed. Digested DNA from transformed 3T3 and rat embryo cells was blotted and hybridized to a variety of likely oncogene probes. New bands of homology were identified when EJ (Ha)-ras was hybridized to both series of blots. Northern dot-blotting of cellular RNA confirmed the elevated expression of a Ha-ras oncogene in transformed mouse and rat cells. Immunoblots of total cellular proteins were similarly reacted against a variety of anti-oncogene product antibodies. Elevated levels of Ha-ras p21 product were resolved from the transformed mouse and rat cells. The oncogenic DNA was unaffected by prior digestion with Eco RI in transformation assays. Efforts are therefore underway to enrich restricted DNA for the oncogenic sequences by preparative DNA digestion, electrophoretic separation, transfection, and molecular cloning. Thus, we hope to shortly characterize a Ha-ras oncogene in a rat tumor, apparently capable of independently transforming normal cells of the same species to a malignant phenotype.

A60 THE INVOLVEMENT OF THE C-MYC GENE IN DIFFERENTIATION OF F9 TERATOCARCINOMA STEM CELLS. Shoshana Segal and Ethan Dmitrovsky. NCI/Navy Medical Oncology Branch, Naval Hospital, Bethesda, MD 20814. Cellular differentiation is a complex and multifactorial process. The mechanisms involved in differentiation are at present poorly understood. C-myc expression has been shown to be down regulated when teratocarcinoma stem cells (F9) were induced to differentiate into non-proliferative parietal or visceral endoderm-like cells. F9 stem cells were transfected with a plasmid construct containing the MoMLV LTR, exon II and III of the human c-myc, and the bacterial neomycin resistance gene. After selection with geneticin (G418) containing media for 10 days, two types of stable transfectants were cloned. The first expressed the transfected human c-myc gene while the second did not. Analyses of these clones included cellular morphology and a variety of differentiation markers induced following treatment with retinoic acid and dibutyryl cyclic AMP. Preliminary data indicate that expression of the transfected c-myc gene may be involved in blocking or delaying differentiation of these cells.

A61 TPA (12-O-tetradecanoylphorbol-13-acetate) INDUCES THE TRANSCRIPTION OF THE c-myc AND c-fos PROTO-ONCOGENES DIFFERENTLY IN MORTAL, IMMORTAL AND TUMORIGENEIC HUMAN UROTHELIAL CELL LINES.
Jan Skouv, Britta Christensen, Jørgen Kieler and Herman Autrup, The Fibiger Institute, Copenhagen, Denmark.

The effect of the skin tumor promoter TPA has been examined in 3 cell lines derived from human urothelium; A) the cell line HU 1752, that is mortal, B) the cell line HCV 29, that is immortal but non-tumorigenic, and C) the cell line HCV 29T, that is a tumorigenic and invasive cell line derived from HCV 29. A single treatment with TPA increased the transcription of the c-fos and c-myc proto-oncogenes of HU 1752 at least 50 fold. The response was transient, and was accompanied by a rapid and transient change in cell morphology. When the HCV 29 was treated with TPA a similar rapid and transient morphological response was observed but the treatment only increased the c-fos transcription. The cell line HCV 29T responded with a weak morphological change but neither c-fos nor c-myc transcription was induced by the TPA treatment. In none of the three cell lines the expression of c-Ha-ras and c-Ki-ras was affected by the TPA treatments.
Thus human bladder epithelial cells responded differently to TPA depending on the growth characteristics and tumorigenecity of the cells.

A62 V-Ha-ras COOPERATES IN CHICKEN ERYTHROID AND MYELOID CELL TRANSFORMATION.

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The ras oncogene is known to be involved in a wide variety of murine and human tumours but its role has not been demonstrated or investigated in the avian system. To explore the effect of ras in primary chicken cells we constructed a replication competent retrovirus containing the v-Ha-ras oncogene.

Infected chicks developed kidney tumours and fibrosarcomas at the site of injection, but not erythroleukemia as was observed in mice (1). Primary chicken embryo fibroblasts showed rapid morphological transformation and displayed characteristic transformation parameters.

In vitro transformation of bone marrow derived erythroid cells with v-Ha-ras yielded erythroid cells with a phenotype resembling that of v-erbB transformed cells(2). Superinfection of these cells with the v-erbA oncogene showed a greatly reduced potential to differentiate as has been observed with erbA in erbB transformed erythroblasts.

We could not directly transform primary myeloid cells with v-Ha-ras. However, superinfection of factor-dependent myb or myc transformed avian myeloid cells with v-Ha-ras resulted in cell growth in the absence of added growth factor (cMGF). These superinfected cells synthesized cMGF as has been described for other oncogenes encoding protein kinases (3).

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2. Frykberg et al. (1983) Cell 32: 227-238.
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A63 MALIGNANT TRANSFORMATION OF A PRENEOPLASTIC HAMSTER EPIDERMAL CELL LINE BY THE EJ c-Ha-ras ONCOGENE, Richard D. Storer, Robert B. Stein, Joseph F. Sina, John G. DeLuca, Henry L. Allen, and Matthews O. Bradley, The Merck Institute for Therapeutic Research, West Point, PA 19486

Recent studies with a Syrian hamster epidermal cell transformation system have indicated that both non-tumorigenic and tumorigenic cell lines can be derived by treatment of primary cultures with chemical carcinogens and subculturing of altered foci of proliferating cells. We have investigated (1) whether activation of endogenous c-Ha-ras genes is associated with the immortalization and/or malignant transformation of primary hamster epidermal cells by chemical carcinogens and (2) whether transfection of the cloned EJ c-Ha-ras oncogene into an established, non-tumorigenic cell line can induce conversion to the malignant phenotype. Transfection studies with genomic DNA from a preneoplastic epidermal cell line immortalized by treatment with N-methyl-'N-nitro-N-nitrosoguanidine and from two neoplastic cell lines transformed by treatment with benzo(a)pyrene failed to detect active transforming genes using the NIH 3T3 cell transformation assay. However, the non-tumorigenic epidermal cell line was rapidly transformed to the malignant phenotype by transfection of the mutated c-Ha-ras oncogene contained in plasmid pEJ. Seven of 9 clones of Eco gpt transformants which were cotransfected with pEJ formed malignant tumors in nude mice and colonies in soft agar. In addition, one of three clones assayed demonstrated metastatic potential in a nude mouse lung colonization assay. These studies demonstrate the ability of a mutated c-Ha-ras gene to rapidly transform an established but non-tumorigenic epithelial cell line.

A64 CHARACTERIZATION OF A GENOMIC CLONE ENCODING THE RAT INSULIN RECEPTOR, Mark A. Tepper, Robert E. Lewis and Michael P. Czech, Department of Biochemistry, University of Massachusetts Medical School, Worcester, MA 01605

Insulin exhibits both metabolic and growth promoting properties by its interaction with specific cell-surface receptors. This receptor has been shown to contain two subunits, α and β, of molecular weight 125K daltons and 90K daltons, respectively. The α subunit contains the insulin binding site, whereas the β subunit contains the tyrosine kinase activity and is autophosphorylated at specific tyrosine residues. How the insulin receptor functions to transmit both metabolic and growth promoting properties remains unclear. In order to further our understanding of how insulin regulates cell growth and metabolism, we set out to clone the rat insulin receptor gene. We have utilized a 47 base oligonucleotide corresponding to a 3' region of the human insulin receptor cDNA, as a probe to screen a rat genomic DNA bacteriophage λ library. Several genomic DNA sequences were isolated that exhibited the same restriction pattern consisting of 4.3, 3.8, 3.2 and 2.5Kb EcoRI fragments. The 47 mer hybridized to the 3.8 Kb fragment. Hybridization of a second oligonucleotide corresponding to the kinase domain of the human insulin receptor cDNA hybridized equally as well to the 4.3Kb EcoRI fragment. Additionally, hybridization of a human insulin receptor cDNA fragment encompassing 600bp of the 3' coding regions hybridized to both the 3.8 and 3.2Kb bands. We are currently performing Southern analysis and DNA sequencing in an attempt to further identify these clones as the rat insulin receptor gene. An additional clone was found that only hybridized to one oligonucleotide. We are presently sequencing this clone.

A65 CLONING AND EXPRESSION OF A TRANSFORMATION-REGULATED LYSOSOMAL PROTEIN, Bruce Troen, Dana Ascherman and Michael M. Gottesman, NIH, NCI, LMB, Bethesda, MD 20892

We have been studying the transformation sensitive regulation of expression of the major excreted protein (MEP) of mouse fibroblast cells. MEP is a secreted lysosomal glycoprotein which is positively regulated by malignant transformation, growth factors (PDGF), and tumor promoters (TPA). Kirsten virus transformed NIH-3T3 (KNIH) cells both produce and secrete up to 50 times more MEP than their non-transformed counterpart NIH-3T3 (NIH) cells. Using an almost full-length cDNA probe from MEP, we have screened 80,000 colonies of a pSV13:cos cosmid library containing NIH-3T3 DNA. Two identical clones containing the MEP gene (pcosMMEP) were isolated. Southern blots of both mouse genomic DNA and cosmid DNA cut with restriction endonucleases, hybridized with the MEP probe, yielded identical banding patterns. Restriction mapping of the cosmid clone revealed that the MEP gene is contained in a region of 15,000 or less base pairs. African green monkey (CV-1) cells were cotransfected with pSV2:neo and pcosMMEP. Stable transformants were selected in media containing G418 (a neomycin analog) and subsquently MXHAT (since pSV13:cos contains the gene for xgpt). ^{35}S-methionine pulse labeling and immunoprecipitation of MEP demonstrated that these stable transformants produce quantities of MEP similar to that found in NIH cells. However, unlike the NIH cells the transfected CV-1 cells exhibit no increase in MEP after exposure to TPA. We are currently transfecting other cell lines with the MEP gene to determine whether the defect in response to TPA lies in the cloned MEP promoter or in the transfected cell line, and to test for regulation of the cloned MEP gene by PDGF and transforming oncogenes.

A66 **EXPRESSION OF A NEW TYROSINE PROTEIN KINASE IS STIMULATED BY RETROVIRUS PROMOTER INSERTION.** **Anna F. Voronova and Bartholomew M. Sefton, The Salk Institute, San Diego, CA 92138**

The LSTRA Mo-MuLV-induced murine thymoma cell line contains approximately 20 fold more phosphotyrosine in protein than do typical hematopoetic cells. This appears to result from the expression of an abnormally high level of a novel tyrosine protein kinase termed p56^{tck}. p56^{tck} is normally expressed at low levels in most, but not all, murine T cell lines, and in normal thymic tissue. The ability of the viral tyrosine protein kinases to induce cellular transformation suggests that elevated levels of p56^{tck} in LSTRA cells could contribute to the malignant properties of these cells.

The major site of in vitro tyrosine phosphorylation in p56^{tck} is present in a tryptic peptide identical to that containing a phosphorylated tyrosine in p60^{src}. A mixture of 32 synthetic heptadecanucleotides containing all possible sequences encoding a portion of this tryptic peptide was used as probe to identify cDNA clones derived from the mRNA for p56^{tck}. Sequence analysis of the full lenth cDNA clones reveals that p56^{tck} is distinct from tyrosine protein kinases described previously. The predominant mRNA for p56^{tck} in LSTRA cells is a hybrid molecule containing 189 nucleotides apparently derived from a MoMLV provirus, including a splice-donor site for the sub-genomic env mRNA of MoMLV, joined to an acceptor site 5 nucleotides upstream of an initiating codon for the p56^{tck} polypeptide. MoMLV promoter insertion is apparently the reason that p56^{tck} tyrosine protein kinase gene is over-expressed in LSTRA cells.

A67 EXPRESSION OF CELLULAR ONCOGENES DURING THE DIFFERENTIATION OF NORMAL BONE MARROW MYELOID PROGENITOR CELLS AND ACUTE MYELOID LEUKEMIA, C.L. Willman, J.K. Griffith, C.C. Stewart and T.B. Tomasi, Univ. of New Mexico, Albuquerque, New Mexico 87131 and Los Alamos National Laboratory, Los Alamos, NM 87545.

To study the alterations in gene expression which occur with normal hematopoietic differentiation, we have developed a bone marrow culture system which expands cells of distinct lineages at unique stages of differentiation. Immature monocytic progenitor cells and more differentiated adherent monocytes and macrophages were isolated from normal murine bone marrow by expansion with CSF-1, the monocytic lineage specific growth factor. Monocytic progenitor cells express high levels of the c-src and c-fos cellular oncogenes which decrease markedly with subsequent normal differentiation. In contrast, levels of c-fms , which encodes the growth factor receptor for CSF-1 on monocytic cells, is expressed at similar levels at each differentiation stage. Initial analysis of oncogene expression in human myeloid leukemias representative of each stage of normal myelomonocytic differentiation has revealed that human myeloid progenitor leukemic cells, capable of monocytic or granulocytic differentiation, express high levels of a 5.0kb c-src mRNA which diminishes with differentiation. These progenitor leukemic cells also express high levels of the c-fms gene which increases with monocytic differentiation but diminishes with granulocytic differentiation. We speculate that the hematopoietic progenitor cell may express multiple growth factor receptors for differentiation along several lineages; and that commitment to one lineage is associated with the selective expression of the growth factor receptor unique to a particular hematopoietic lineage.

A68 AMPLIFICATION OF RNA AND DNA SPECIFIC FOR ERB B IN UNBALANCED 1;7 CHROMOSOMAL TRANSLOCATION, Gayle E. Woloschak, Gordon Dewald, Robert Kyle, and Philip Greipp, Mayo Clinic, Rochester, MN 55905.

Previous work has established the presence of an unbalanced chromosome abnormality [+der(1),t(1;7)(p11;p11)] in some therapy-associated myeloproliferative disorders. Recently the EGF receptor has been found to reside at 7p11. Using a probe specific for erb B oncogene, which encodes a truncated form of the EGF receptor, we examined RNA and DNA derived from bone marrow and peripheral blood mononuclear cells from patients with an unbalanced 1;7 translocation. DNA-excess slot blot hybridization to 5'-32P-labelled cellular RNA revealed greater than twenty-fold enhancement in accumulation of mRNA specific for erb B in both peripheral blood and bone marrow cells when compared to normal controls. In addition, a three-fold enhancement of K-ras mRNA accumulation was detected. Expression of other genes such as actin, N-ras, myc, src, B-lym, and 26 other genes was not found to be enhanced. Increased erb B expression was not apparent in mononuclear cells from patients with other hematologic disorders such as CLL, Hodgkin's disease, or lymphoma. Southern blot analysis of Bam HI, Eco RI and Hind III cleaved DNA from one patient bearing the 1;7 translocation revealed that erb B gene was amplified at least twenty-fold in both bone marrow and peripheral blood mononuclear cells, while control levels of K-ras and N-ras genes were detected. In addition, Southern blots also demonstrated the presence of unusual erb B bands in our patient indicative of erb B gene rearrangement. Our data suggest that t(1;7)(p11;p11) translocations are specifically associated with amplification of erb B DNA and RNA sequences.

A69 DETECTION OF ACTIVATED ras p21 PROTEIN IN CELL EXTRACTS USING ANTIBODIES SPECIFIC FOR THE TWELFTH AMINO ACID, Gail Wong, Norman Arnheim, Robin Clark, Bob Milley, Peter McCabe, Mike Innis and Frank McCormick. Dept. of Human Genetics, Cetus Corporation, Emeryville, CA 94608

Using peptide immunogens, we have been able to raise antibodies that distinguish between different forms of p21 according to the amino acid at the twelfth codon. Peptides spanning this codon (residues 6-16) were synthesized with either glycine, valine, serine, arginine, aspartate, alanine, or cysteine at residue 12. Normal p21 contains glycine at position 12; the other residues are those that can be generated by a single base change in codon 12 and may therefore be the activating mutations most likely to occur in human cancer. To screen the antisera, we used site-directed mutagenesis to create corresponding mutants in v-Ki-ras p21 expressed in E. coli.

The peptide immunogen that contained glycine at position 12 failed to generate antibodies capable of reacting with p21 protein. Peptides with serine, arginine, aspartate, or valine at position 12 generated sera with specificity for p21 mutants with these amino acids at position 12. For example, antibodies raised against the serine-containing peptide were able to immunoprecipitate v-Ki-ras p21 with serine at 12, but not p21 with glycine, valine, arginine, aspartate, or cysteine at this position. Slight cross-reactivity to the alanine mutants was detected. Peptides with alanine at position 12 cross-reacted with all forms of p21, including glycine-12, but the cysteine-containing peptide failed to generate anti-p21 antibodies. Anti-p21-serine, anti-p21-valine, anti-p21-arginine and anti-p21-aspartate sera have been used to detect corresponding mutant forms of p21 in mammalian cells.

A70 **PRIMITIVE HEMOPOIETIC PROGENITOR CELLS ARE TARGETS FOR ABELSON MURINE LEUKEMIA VIRUS (A-MuLV), Peter M. C. Wong, Siu-Wah Chung, Eric Raefsky, Connie J. Eaves, Arthur W. Nienhuis, National Institutes of Health, NHLBI and NCI, Bethesda, MD and NCI, Bethesda, MD 20892, and B.C. Cancer Research Center, Vancouver.**

We have established an in vitro system with which to examine the ability of A-MuLV to infect early hemopoietic progenitor cells. Adult mice were treated with 5-fluorouracil (5-FU) at a dose of 150 mg/kg. Four days later, single cells from spleens of these animals were placed in standard clonogenic methylcellulose stem cell culture. Another 6-8 days afterwards, blast cell colonies consisted of about fifty cells were observed. They were then plucked and individually exposed to A-MuLV, also containing the Moloney helper virus in the viral stock, on top of an irradiated feeder. Four out of ten such colonies resulted in factor independent cell lines two months after infection. Subsequently, more detailed analysis by means of replating half of each colonies with the other half subjected to infection indicated that there is a correlation between generation of cell lines and the formation of mixed colonies during replating. Southern analysis on two lines using v-abl probe suggested random viral integration and less than three cells were originally infected. In order to eliminate the possible effect of the Moloney helper also present in the A-MuLV, we sought to generate helper-free A-MuLV. P160 proviral A-MuLV DNA was transfected into ψ-2 cells and helper-free A-MuLV virus with a titer of more than 5 x 10^5 FFU/ml was generated. Preliminary studies using this viral stock revealed that cell lines may also be generated. Taken together, these data suggest that early hemopoietic progenitor cells are targets for the A-MuLV.

A72 COMPARISON OF ONCOGENE EXPRESSION IN NORMAL RAT THYROID WITH SPONTANEOUS AND RADIATION INDUCED RAT THYROID CARCINOMA. Zain, S., Watkins, R., Kaminsky, S., Macara, I., and Mulcahy, T.

The hypothesis that oncogene expression occurs as a step in radiation carcinogenesis, was evaluated using a rat thyroid carcinogenesis model system. This system capitalizes on the exquisite sensitivity of thyroid epithelium to the carcinogenic effects of ionizing radiation. Normal thyroid tissue, tissue from spontaneously developed rat thyroid carcinoma and cells derived from radiation induced thyroid carcinoma in early passage were used as a source of tumor material. Comparison between the genomic DNA, mRNA and proteins between these tissues revealed the expression of a set of oncogenes differing qualitatively (myc, ras^K) and quantitatively (myc) from the normal. In addition, the DNA from the tumor tissue gives indications of genomic rearrangements. DNA mediated gene transfer assay using NIH 3T3 cells gave higher number of foci compared to the normal tissue DNA.

The involvement of oncogene expression in TSH induced proliferation was investigated utilizing a normal, differentiated in vitro thyroid cell model (FRTL-5). Following induction by TSH there is marked increase in the amount of Ras^K mRNA identified relative to the non-induced cells. Analysis of other oncogene expression following thyrotropin stimulation in other proliferative modulation will be described. The involvement of hormone induced proliferation and its associated oncogene expression in the process of radiation carcinogenesis of thyroid epithelial cells will be discussed.

A74 AMPLIFIED ONCOGENES AND CHROMOSOMAL ABNORMALITIES IN TUMOR CELLS

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Regulatory or structural alterations of cellular oncogenes have been implicated in the causation of various cancers. Amplification of cellular oncogenes can augment their expression by increasing the amount of DNA template available for the production of mRNA. It appears that amplification of certain oncogenes is a common correlate of the progression of some tumors and also occurs as a rare sporadic event affecting various oncogenes in different types of cancer. Amplified copies of oncogenes may or may not be associated with chromosomal abnormalities signifying DNA amplification: double minute chromosomes (dmin) and homogeneously staining chromosomal regions (HSR:s). Amplified oncogenes, whether sporadic or tumour type-specific, are expressed at elevated levels, in some cases in cells where their diploid forms are normally silent. Increased dosage of an amplified oncogene may contribute to the multistep progression of at least some cancers.

We have discovered and characterized several cases of oncogene amplification in human tumor cell lines and human tumors. The c-myb gene is expressed only in immature hematopoietic cells and in the COLO 201/205 colon carcinoma cells where it is amplified. The amplified copies of c-myb reside in two marker chromosomes that have envolved from chromosome 6 by complex chromosomal rearrangements. This suggests that c-myb was amplified in situ in a segment that became translocated to the marker chromosome without the extrachromosomal intermediate form of dmin. No HSR:s can be discerned at the site of c-myb amplification, but there is an enhanced frequency of sister chromatid exchanges (SCE:s) at this site. The overall rate of SCE:s may be related to the dose level of the myc-class oncogenes in malignant cells. The myc oncogenes are often found amplified in lung cancer. Amplification of c-myc (up to 30-fold) is also detected in some fresh AML (M2) cells containing dmin. This suggests that clonal evolution of some leukemia cell populations may involve selection for increased dosage of oncogenes.

Sequences of the short arm of chromosome 2 containing the N-myc oncogene at 2p23-p24 are often involved in DNA amplification in neuroblastomas. We have searched for other genes in this chromosomal region. We have found that the human ornithine decarboxylase (ODC) sequences map to chromosome 2, region 2p23-pter, and are expressed as a mRNA species of 2.2 kb in several tumor cell lines. However, the ODC sequences are not coamplified with the N-myc oncogene. Our further experimental studies should clarify, whether N-myc is included in the amplicon resulting from a selection pressure for overexpression of ODC in neuroblastoma cells.

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A75 INTERLEUKIN 2 RESPONSIVENESS OF IMMATURE T-CELL COLONY-FORMING CELLS (T-CFC) FROM PATIENTS WITH ACUTE T-CELL LYMPHOBLASTIC LEUKEMIAS.

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T-cell colony-forming cells (T-CFC) from 13 of 16 patients with T-ALL generated colonies in methylcellulose in the absence of added growth factors or mitogenic stimulation. As previously described, these colonies were composed of immature T-cells displaying the same karyotypic abnormalities as fresh leukemic cells. Biochemically purified (bIL2) and recombinant IL2 (rIL2) without any mitogen enhanced colony growth from both unfractionated and blast-enriched cell fractions in patients with a relatively low (less than 50 colonies/$5x10^4$ cells) plating efficiency. However, dose-response experiments revealed that the optimal dose of rIL2 needed to enhance colony growth varied from patient to patient. Anti-IL2 (DMS1) and anti-IL2-receptor (anti-Tac) moAbs inhibited both spontaneous and rIL2-induced colony formation in a dose-dependent manner. Direct staining of fresh leukemic cells with anti-Tac revealed less than 10% positive cells in all but 2 patients. However, cell incubation in the absence of growth factors or mitogens, for 2-48 hr resulted in an increase of Tac^+ cells. These observations indicate that a subset of immature T-CFC from T-ALL patients display functional IL2-receptors. In addition, our findings strongly suggest that colony formation in the absence of added growth factors is mediated through a spontaneous activation of the IL2/IL2-R system.

A76 IMMUNODIAGNOSTIC AND THERAPEUTIC POTENTIAL OF A MONOCLONAL ANTIBODY TO A c-Ha-ras LINKED HUMAN TUMOR-ASSOCIATED ANTIGEN.

Robert S. Ames, Harvey I. Pass, Howard M. Lee, Patrick J. Scannon, and Jack A. Roth. NCI Surgery Branch NIH Bethesda, MD, George Washington University, Washington D.C., and Xoma Corp., Berkeley, CA.

An IgG_1 murine monoclonal antibody (45-2D9) was generated against a c-Ha-ras NIH-3T3 tertiary transfectant (45-342). Indirect radioimmunoprecipitation and immunoblot analysis revealed that this antibody binds to a 74K Mr glycophosphoprotein that is distinct from p21. Immunoperoxidase staining demonstrated binding primarily to adenocarcinomas (18/37) but not to other oncogene or virally transformed NIH-3T3 cells or normal human tissues. ^{125}I-labeled 45-2D9 localized to subcutaneous tumors and pulmonary metastases expressing gp74 but not to tumors lacking the antigen. 45-342 tumor to normal tissue ratios of $\geq$10:1 were achieved with ^{125}I-45-2D9 while ratios of 1:1 were obtained with the control ^{125}I-labeled MOPC-21. An immunotoxin was prepared by conjugating the A chain of ricin to the 45-2D9 monoclonal antibody. Binding of the immunotoxin was equivalent to the unconjugated antibody in a radiolabeled competition binding assay. In vitro the immunotoxin was two logs more effective than an immunotoxin directed at determinants not expressed on 45-342 cells in inhibiting protein and DNA synthesis of fresh 45-342 tumor cells. In a colony forming assay 3 logs of cytotoxicity were demonstrated. Monoclonal antibodies produced against oncogene transformed cells may prove useful in targeting radioisotopes and toxins as an aid in the diagnosis and therapy of human tumors.

A77 THE STRUCTURE OF DOUBLE MINUTE (DM) CHROMOSOMES ANALYSED BY PULSED FIELD GRADIENT (PFG) GEL ELECTROPHORESIS. P. Borst, A.M. Van der Bliek, T. Van de Velde-Koerts and E. Hes. The Netherlands Cancer Institute, Amsterdam, The Netherlands.

Many tumour cells contain amplified DNA in the form of DMs or Homogeneously Staining Regions (HSRs). To study the structure of this DNA, we have lysed and deproteinized cultured cells in agarose blocks, digested the DNA within the blocks with various infrequently cutting restriction endonucleases and separated the resulting fragments by PFG gel electrophoresis, as used for the analysis of trypanosome choromosomes in our lab (1). Undigested mammalian DNA does not enter the gel, neither do DMs, presumably because they are circular. Singly cut DMs from four different cell lines migrate in a compression zone high in the gel, indicating a size over 1500 kb. Our data on the mouse adrenocortical Y1-DM (cKi-ras amplification) line suggests that there is a homogeneous amplicon of 900 kb (calibrated with phage lambda oligomers); the Y1-HSR line has a more complex amplicon. The amplified DHFR gene in the DMs of the mouse EL4/12 line appears to be present in units of 275, 350 and 475 kb; attempts to establish the relation between these units by partial restriction enzyme digestion have failed. Approximate sizes of the amplicons of several other lines have been determined and will be presented.

(1) L.H.T. Van der Ploeg et al., EMBO J. 3 (1984), 3109-3115.

A78 **MOLECULAR BIOLOGY OF t(14;18) TRANSLOCATIONS IN HUMAN FOLLICULAR LYMPHOMAS** Michael L. Cleary, Naomi Galili and Jeffrey Sklar, Stanford University, Stanford, CA 94305

A fragment of DNA containing the crossover point between chromosomes 14 and 18 was cloned from the tumor cells of three patients with follicular lymphoma containing a t(14;18) translocation. Nucleotide sequence analysis of the breakpoint DNA in each case revealed that the break in chromosome 14 occurred near J4 or J6 of the non-functional immunoglobulin heavy chain allele. These findings and other structural similarities of the breakpoints with the functional D-J joint in each lymphoma suggest that D-J recombination enzymes played a role in the mechanism of the t(14;18) translocation. Genomic hybridization analyses showed that 60% of follicular lymphoma DNA samples contained breakpoints which clustered within a small region of chromosome 18. DNA probes flanking this breakpoint cluster region detected transcription products in a variety of B-lineage lymphomas, leukemias and lymphoblastoid cell lines. cDNA and genomic sequencing studies suggest that this major breakpoint cluster region lies at the 5' end of a transcriptional unit on chromosome 18.

Approximately 40% of follicular lymphomas do not have chromosome 18 breakpoints which lie within the cluster region described above, yet they contain a t(14;18) karyotypic abnormality. Breakpoint DNA was isolated from the cells of such a lymphoma and a DNA probe was constructed which detects chromosome 18 DNA rearrangements in most of the 40% whose breakpoints were previously undetectable on Southern blot hybridizations. The combined use of these two cluster region probes may prove diagnostically useful for detecting the vast majority of t(14;18) translocations in genomic Southern blots on DNA extracted from lymphoma biopsy specimens.

A79 Chromosomal alterations in early stages of mouse skin papilloma development. C.J. Conti, C.M. Aldaz, A.J.P. Klein-Szanto, J.F. O'Connell and T.J. Slaga, Univ. of Texas System Cancer Center, Science Park-Research Division, Smithville, Texas.

The mouse skin is probably the most extensively studied model of chemical carcinogenesis. However, one aspect that has not been explored in this model, is the role of chromosome alterations in tumor development. For this reason, with methods recently developed in our laboratory, we have studied the chromosome constitution of papillomas and carcinomas induced by two-stage protocol (DMBA-TPA).

After 10 weeks of promotion approximately half of the papillomas were purely diploid but the rest of the papillomas showed aneuploid clones and in one case an hyperdiploid clone of 41 chromosomes constituted the stem line. By 20 weeks of promotion almost every papilloma presented chromosome gains in several clones and at 40 weeks most of the stem lines of the papillomas were hyperdiploid (41-44 chromosomes). Double blind studies showed a positive correlation between the level of aneuploidy and atypia. Twenty squamous cell carcinomas obtained with the same protocol were aneuploid with a hyperdiploid mode of 41-48 chromosomes and with a secondary mode of metaphases with doubled chromosomal complement. G-banded preparation of these tumors showed an apparent non-random involvement of chromosome #2.

It appears from these results that benign tumors show from early stages, a high level of chromosomal unstability that may be mechanistically related to the progression to malignant tumors. (Supported by grants CA 34962, CA 34521 and CA 38863).

A80 INHIBITION OF TRANSFORMED HAMSTER BONE MARROW CELLS BY A FACTOR PRODUCED BY ADHERENT CELLS OF LONG TERM BONE MARROW CULTURES, C.E. Eastment, F.W. Ruscetti, S. Ruscetti and L. Haiber Am Red Cross and NCI, Bethesda, MD and FCRF-NCI, Frederick, MD

Several transformed cell lines, termed ARC lines, have recently been isolated and cloned from Syrian hamster long term bone marrow cultures. The lines were derived from spontaneously transformed cultures after alteration of the original culture conditions. Conditioned medium from these lines contains large number of type C viral particles. The virus can be detected in a nonactivated state in normal hamsters, transforms fresh hamster bone marrow in vitro, and also produces leukemia when injected into newborn mice. Each of the ARC lines has produced numerous clones which are morphologically similar to the parent line, but exhibit variable responses to several histochemical stains as well as an inhibitory factor that is produced by the adherent cells from hamster long term bone marrow cultures (Eastment and Ruscetti, BLOOD 65:736, 1985). The factor is active under both cell-free and serum-free conditions. In normal hamster long term bone marrow cultures the addition of adherent layer conditioned medium (ALCM) containing this factor inhibits the erythropoietin-stimulated terminal maturation of erythroid progenitors. Addition of this ALCM to actively growing, transformed ARC cells inhibits their proliferation by as much as 70% for some clones while other clones demonstrate little or no inhibition in the presence of ALCM. The inhibitory factor appears to be a chymotrypsin-sensitive protein with a molecular weight of over 10,000 daltons. Efforts are presently underway to further characterize this protein.

A81 MOLECULAR-CYTOGENETIC STUDIES OF THE 11;22 TRANSLOCATION, B.S. Emanuel[1], C.A. Griffin[1], C. McKeon[2], D. Stehelin[3], J. Ghysdael[3] and M.A. Israel[2], Children's Hosp. of Phila.[1], Phila., PA 19104, NIH[2], Bethesda, MD 20205, and Institut Pasteur[3], France

A site specific translocation involving chromosomes 11 and 22, t(11;22)(q23-24;q11-12) has been observed in Ewing's Sarcoma (ES), a tumor of unknown cellular origin and in two tumors of neural origin, peripheral neuroepithelioma and Askin's tumor. A similar translocation, t(11;22)(q23;q11) has been observed in the constitutional karyotype of numerous unrelated normal individuals. Constitutional translocation carriers do not develop 11;22 related tumors, whereas individuals who have the tumors have normal constitutional karyotypes. Using chromosomal *in situ* hybridization we have begun to examine the 11 and 22 breakpoints in an attempt to detect differences between these 11;22 translocations at the molecular level. Using a probe for the constant region of the immunoglobulin lambda light chain which maps to 22q11 we have determined that the constitutional 22q11 breakpoint is more proximal than the ES or NE breakpoint. In constitutional carriers most of Cλ translocates to the $11q^+$ whereas in ES or NE most of Cλ remains on the der(22). Using a probe for the c-*ets* oncogene which maps to 11q23 we have examined one NE cell line and one constitutional t(11;22). Our data suggest that *ets* translocates to the $22q^-$ chromosome in the constitutional t(11;22) but remains on the involved chromosome 11 in NE. Thus, we have detected differences between these translocation breakpoints with molecular cytogenetic techniques. The relevance of these differences to the cellular phenotype, neoplastic versus normal, will be discussed.

A82 v-ras^H TRANSFECTION MIMICS AND BYPASSES ESTROGEN-INDUCED TUMOR PHENOTYPE OF A HUMAN BREAST CANCER LINE, Edward Gelmann, Robert Dickson, Karen Huff, Susan Bates, Cornelius Knabbe, Diane Bronzert, Attan Kasid and Marc Lippman, Medicine Branch, National Cancer Institute, Bethesda, Maryland 20892

The hormone-responsive human breast cancer cell line, MCF-7, responds to physiologic levels of exogenous estrogen by increasing cellular growth rate, by secreting growth-promoting peptides, and by manifesting tumorigenesis in the nude mouse. We have transfected the v-ras^H gene into MCF-7 cells to investigate the influence of an activated oncogene on the cell's hormone-dependent properties. Stably transfected MCF-7 cells (MCF-7_{ras}) integrated several copies of the v-ras^H gene, had 5-8 times the level of ras mRNA as in control cells, and had detectable phosphorylated p21. MCF-7_{ras} cells displayed accelerated growth in vitro and resistance to growth inhibition by antiestrogens. The transfected cells were tumorigenic in the absence of estrogen in 85% of inoculated oophorectomized nude mice. In vivo MCF-7_{ras} cells injected into the mammary fat pad of nude mice stimulated tumor growth of MCF-7 cells injected at a remote site. Moreover the MCF-7_{ras} cells secreted growth-promoting factors similar to some of the growth factor peptides induced by estrogen treatment of control MCF-7 cells. Conditioned medium prepared from MCF-7_{ras} cultures as compared with control cultures contained 3-4 fold elevated levels of radioreceptor assayable TGF-α and total transforming growth factor activity as assayed by anchorage-independent growth of NRK cells. A single peak of TGF-α-like activity was eluted at an apparent MW of 30,000 from acid gel chromatography of MCF-7_{ras} condition medium. TGF-β secretion was increased in MCF-7_{ras} cells 5-fold over control. Also, secretion of immunoreactive IGF-I was augmented 3-4 fold in MCF$_{ras}$ cells. Ras gene activation can bring about phenotypic and tumorigenic changes which may also be induced by estrogens in human breast cancer cells. However, the cells retain the capacity to bind estrogen and respond to estrogens as shown by induction of the progesterone receptor. Thus ras gene transfection bypasses estrogen activation of the transformed phenotype and induces that phenotype via a pathway independent of hormone induction.

A83 THIOPROLINE REVERSE MODIFICATION CONDUCES TO MORE SOCIAL AND TRANQUIL CULTURE BEHAVIOR, Mario Gosálvez, Mariaflor Blanco, Rafael García-Cañero and Mercedes Bueno, Research Sector, Department of Medicine, Clínica Puerta de Hierro, 28035 Madrid, Spain

Thioproline, a new biological response modifier (The Lancet, p. 1108, May, 1983; Neoplasm 29, 535, 1982; Amer. J. Vet. Res. 45, 2162, 1984), induces reverse modification in the less malignant compartment of HeLa cell cultures, conducing to a more social and tranquil behavior of the whole culture. This affirmation is supported by the following observations in treated cultures with respect to control: a) decrease of saturation density, b) enlargement, flattening and reduced cell adhesiveness, c) decreased transport and macromolecular synthesis, d) increase in cell junctions and in cytoskeletal proteins, e) increased mitotic and intermitotic time, f) increased secretion and pseudopodia, g) increase in rapid zig-zag movement and spatiotemporal ranges, and h) higher population of benign leader cells and lower population malignant "leader cells". (Supported by the CAIC y T no. 1475/82 (1985).

A84 MOLECULAR GENETIC STUDIES OF THE 9;22 TRANSLOCATION OF ACUTE LYMPHOCYTIC LEUKEMIA Constance A. Griffin[1], Jan Erikson[2], Peter C. Nowell[3], Mauro Valtieri [2], Carlo Croce[2] and Beverly S. Emanuel.[1] Children's Hospital of Philadelphia[1], Wistar Institute[2], Dept. of Pathology and Laboratory Medicine, University of PA[3]

The typical Philadelphia chromosome (Ph) of chronic myelogenous leukemia (CML) is formed by reciprocal translocation between the long arms of chromosomes 9 and 22[t(9;22)(q34;q11)]. The breakpoints on chromosome 22 fall within a 5.8 kb "breakpoint cluster region" (bcr). The same translocation is also found in about 10% of cases of acute lymphocytic leukemia (ALL). Although cytogenetically the breakpoints on 9 and 22 appear to be the same as in CML, detailed knowledge about the molecular biology is not yet available. We have studied 2 cases of Ph^+ ALL by in situ hybridization with probes for the Cλ immunoglobulin gene and bcr region. We have found that the breakpoint on 22 is between the λ and bcr genes proximal to the breakpoint in CML. In both patients the λ probe hybridized to the normal 22 and Ph chromosomes, but the proportion of hybridization to distal Cq (which includes $9q^+$) was no higher than in control cells, suggesting that Cλ does not translocate. In contrast, in both patients the bcr probe hybridized to the normal 22 but not to the Ph chromosome, and hybridization to distal Cq (which includes $9q^+$) was twice that of normal, suggesting that bcr does translocate. Southern blot data indicates that in contrast to what is seen in CML, bcr is not rearranged. We are presently studying the role and chromosomal location of the abl oncogene in these 9;22 ALL translocations.

A85 THE c-abl ONCOGENE IN CHRONIC MYELOGENOUS LEUKEMIA G. Grosveld, A. Hermans, D. Bootsma, A. de Klein, N. Heisterkamp[a], K. Stam[a] and J. Groffen[a]. Dept. Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands and (a) Oncogene Science Inc., Minola, N.Y., USA.

Chronic myeloid leukemia (CML) is characterized by the presence of the Philadelphia (Ph') chromosome in the leukemic cells of 96% of all CML patients. The Ph' chromosome (22q-) is the result of a reciprocal translocation between chromosome 22 and chromosome 9, t(9q34,22q11). Previously we described the localization of the human c-abl oncogene on chromosome 9 and demonstrated its translocation to the Ph' chromosome in CML patients. The cloning and analysis of breakpoint fragments revealed that the breakpoints on chromosome 22 all cluster in a very limited area, the breakpoint cluster region, bcr.
Breakpoints on chromosome 9, however, are scattered over a large area which may vary from 14 kb up to more as 100 kb upstream of the v-abl homologous sequences of the c-abl gene. The detection of a chimeric mRNA (5' bcr and 3' abl sequences) in the leukemic cells of CML patients and the cloning of chimeric cDNAs from a CML derived cell line K562 strongly indicate that bcr and c-abl coding sequences are linked by RNA splicing, independent from the distance between the two genes on the Ph' chromosome.

A86 LYMPHOMA GROWTH FACTOR SECRETED BY MURINE T LYMPHOMAS AND BY HUMAN ALL(T) CELLS. Martin Haas, Ph.D., Martin I. Mally, Ph.D., Jakob Bogenberger, Ph.D., Department of Biology/Cancer Center, University of California, San Diego, La Jolla, CA 92093, U.S.A.

Autostimulatory growth of murine X-ray-induced T-cell lymphomas (TCL) is mediated by a growth factor which we have called Lymphoma Growth Factor (LGF). All newly established TCL lines secreted LGF and were dependent on it for growth. We have established specific assay systems for LGF and have shown that LGF differs from the known interleukins, interferons, and TGFs, and appears to represent a novel growth factor. Northern blots containing mRNAs extracted from LGF-secreting TCL cells were hybridized with probes specific for interleukins -1, -2, or -3. These experiments showed that TCL cells did *not* synthesize IL-1, -2, or -3 specific mRNAs, and that LGF mRNA differs from the known interleukin mRNAs. Xenopus oocytes injected with mRNA extracted from LGF-secreting cells synthesized *biologically active* LGF. Molecular cloning of the genes coding for LGF is in progress.

LGF-dependent TCLs were analyzed for insertions or deletions (e.g. promoter insertions) in the c-myc and the c-pim domains, using Southern blotting. The karyotypes of TCL cells were also determined. No promoter insertions in the vicinity of myc or pim were detectable; neither trisomy of chromosome #15(myc) nor #17(pim) were found. Our findings suggest that activation of an autocrine loop is involved in the initiation of the lymphoma state, while promoter insertion would constitute a later step in the progression of TCLs.

Newly established human ALL(T) lines were shown to proliferate via an autocrine mechanism and secrete biologically active LGF as assayed in our tests for LGF activity.

A87 T-cell receptor gene rearrangement in human ATL samples. Michio Hagiya*, Toshio Hattori+, Masao Matsuoka+, Kiyoshi Takatsuki+ and Hitoshi Sakano* *Dept. OF Microbiology and Immunology, Univ. of Calif. Berkeley, CA 94720, and +2nd Dept. of Internal Medicine, Kumamoto Univ. School of Medicine, Kumamoto, Japan.

We have analyzed 50 different human ATL samples for T-cell receptor gene rearrangement. It was found that one of the V_β genes cloned from an ATL patient Imabayashi, $V_{\beta\text{-IM}}$, is very frequently rearranged in other ATL samples (>50%). It was also noticed that the $V_{\beta\text{-IM}}$ always gave a secondary rearrangement associated with the functional $V\text{-}D\text{-}J_{\beta\text{-IM}}$ joining in the same ATL cell. These two rearranged structures have been cloned and their nucleotide sequences will be compared.

5'-sequences of several different V_β genes were analyzed for possible transcriptional control elements. A conserved heptamer, TCTTTCT, was found about 200bp upstream of a start codon ATG. In order to study if this heptamer is responsible for tissue specific expression of T-cell receptor genes, the heptamer was deleted with an exonuclease Bal31, and the deleted gene was introduced into T-cell lines. RNA transcription was analyzed in both transient and stable transformants.

A88 CELLULAR CHANGES IN HUMAN FIBROBLAST GROWTH PROMOTING FACTOR-HFGPF RECEPTOR DURING TRANSFORMATION INTO TUMORIGENIC CELLS. Anwar A. Hakim and Charles E. Joseph. Loyola Medical Center. Maywood. Illinois 60153. & Univ. Southern California. Los Angels.CA.

The rate and extent of proliferation of normal somatic cells are governed by hormone-like growth factors and are under the influence of a still poorly defined "biological Clock" which induce their senescence. Neoplastic cells seem to have escaped such a dual regulation since they have an unlimited life span, exhibit reduced growth factor requirements and often produce their own mitogenic signals. The present studies examined the effects of a fibroblast growth promoting factor-HFGPF (Hakim, Experientia 34,1515,1978) on human skin fibroblast HSF (Hakim, J. Suppramol. Structures, Suppl. 3,224,1979), human malignant melanotic (HMMC-ShA, HMMC-WJP) and amelanotic (HMMC-SR) melanoma cells (Hakim, Neoplasma 24, 81,1977; Res. Exp. Med. 18o, 99, 1982).The results indicate that HFGPF-like substance is impottant in the progression and initiation of DNA-synthesis. HFGPF is required by HSF cells specifically in the mid GoS. The requirement for HFGPF is diminished in HMMC-SR, and is eliminated in HMMC-ShA and MMC-WJP cells. One cause of this decreased requirement is that the melanoma cells produce " tumor cell growth factors-TCGF, which takes the place of HFGPF . Media conditioned by HMMC-Sha and HMMC-WJP were able to block HFGPF binding by HMMC-SR and HSF cells. Whereas, media similarly prepared from HMMC-SR did not replace HFGPF or block binding. HMMS-SR have specifically diminished their HFGPF requirement. After passage in Nude mice, these cells totally lose their HFGPF requirement. The number of HFGPF binding sites n the cell surface is progressively decreased during their changes to tumorigenic cells.Among these changes is the conversion of normal gene(prot-gene) to transforming gene (Oncogenes).

A89 A UNIFIED MODEL CONNECTING PROLIFERATION-SPECIFIC AND DIFFERENTIATION-ASSOCIATED CHROMOSOME BREAKPOINTS IN HUMAN NEOPLASIA, S. Heim and F. Mitelman, Department of Clinical Genetics, University Hospital, S-221 85 Lund, Sweden

Based on the distribution of interchromosomal rearrangements in human neoplasia, the tentative conclusion was drawn that one of the two breakpoints in a cancer-associated reciprocal translocation is more important to the proliferative, neoplastic process per se, the other to differentiation-related aspects of the cell type in question. We have attempted to test the intrinsic consistency of this model by interconnecting breakpoints which are known to participate in cancer-associated translocations. The six breakpoint regions informative in this respect were all compatible with the proposed model. An additional 18 breakpoints could be linked to the first six. Of the 12 breakpoint regions classified as proliferation-associated by this approach, eight turned out to be located in bands which also contain cellular oncogene sites. On the other hand, only one of the 12 differentiation-associated regions coincided with a known oncogene location. Thus, there seemed to be a marked correspondence between proliferation-related chromosome segments as predicted by the model, and c-onc genes. The same model, although originally based on consistent interchromosomal rearrangements, has also been expanded to tentatively incorporate and help explain the majority of intrachromosomal aberrations in human neoplasms.

A90 REGULATION OF THE EGF RECEPTOR IN SQUAMOUS CELL MALIGNANCIES, Fred Hendler, C. Sue Richards, Alice Shum, and Brad Ozanne, University of Texas Health Science Center, Dallas, TX 75235.

Evidence is rapidly accumulating which suggests that the EGF receptor plays an important role in the development and regulation of epidermoid malignancies. We have shown that the EGF receptor is increased in squamous cell carcinomas in tissue culture and in most if not all human squamous tumor biopsy specimens. The increase in receptor observed is as much as 50-fold greater in HN-5 cells than cultured keratinocytes. The receptor values in over 70 human tumor biopsy specimens have ranged from 1.5 to 20-fold greater than normal skin. Using human EGF receptor gene probes we observed that the EGF receptor gene is always amplified in cultured cells and 9 of 9 biopsy specimens. The cell lines with the greatest amount of gene amplification synthesize the most amount of EGF receptor mRNA and appear to have the greatest number of receptors. The receptor gene is rearranged in at least 3 cell lines, A431, HN-1, and HN-2, with synthesis of a similar 2.8 Kb transcript. Many of the squamous tumor cell lines with increased EGF receptors are EGF independent. We have shown that these cell lines synthesize TGF_α, an EGF analogue with high affinity for the EGF receptor, and contain TGF_α mRNA. The TGF_α gene is not amplified in these cell lines. Thus, the increased EGF receptor may be functional in epidermoid tumors and the increase in receptor content may be the mechanism by which autocrine regulation is achieved.

A91 PRODUCTION AND IMMUNOHISTOLOGICAL ANALYSIS OF ONCOPROTEIN-SPECIFIC MONOCLONAL ANTIBODIES. Hermann Herbst, Hans-Christoph Kratzsch, Roland Schwarting, Johannes Gerdes, and Harald Stein. Institute for Pathology, Klinikum Steglitz, Free University Berlin, Berlin, West Germany.

Segments of a variety of viral and cellular oncogenes (fos, mos, H-ras, K-ras, myc, sis, src and others) with up to 25 kd coding capacity were expressed as trpE fusion proteins after subcloning into expression vectors of the pATH series. Employing these fusion proteins as immunogens, monoclonal antibodies were generated and screened using cytospins of cell lines expressing the respective oncogenes at high levels as well as non-expressing cells and the immuno-alkaline phosphatase (APAAP) method. Subsequent biochemical analyses verified the reactivity with oncogene encoded products. In addition, antibodies were obtained detecting determinants shared by oncogene products and other proteins. One example is antibody Ber-sis 2 which stains normal cells in mitosis as well as all v-sis expressing cells. The reactivity of these antibodies on a panel of cell lines and various proliferating human tissues will be shown and the potential of these reagents for the histomorphological analysis of human tumors will be discussed.

A92 PRODUCTION OF HUMAN ANTIBODIES TO BOMBESIN AND TETANUS TOXOID BY IN VITRO IMMUNIZATION, May-Kin Ho, Neelam Rand, Kenneth P. Kato, James H. Murray, and Harvey Rabin, E.I. Dupont deNemours & Co., Inc, No. Billerica, MA 01862

To allow the production of human monoclonal antibodies (MAb) to tumor antigens, a procedure was developed to sensitize human B lymphocytes in vitro against bombesin conjugated to tetanus toxoid (BTT). Bombesin-like substances are found in cells of small cell lung tumors, brain, fetal lung, and gastrointestinal tract. Human spleen cells were separated on nylon wool columns into nylon-nonadherent (NA) and nylon-adherent (A) fractions. The NA cells were mixed with equal numbers of A cells, 7.5μg/ml lipopolysaccharide, 10% human AB serum, 20% culture supernatants from PHA-activated lymphocytes (LCS) and various concentrations of BTT. After 6 days of culture, the splenocytes were fused with NS-1 mouse myeloma cells. MAb specific for bombesin or tetanus toxoid were generated in an antigen-dependent manner using this approach. Binding of human anti-bombesin MAb to BTT in ELISA was inhibited by BTT, bombesin-conjugated to thyroglobulin, and unconjugated bombesin, but not by TT or thyroglobulin. In contrast, the binding of anti-TT MAb to BTT was only inhibited by BTT and TT. For optimal anti-TT responses, nylon wool separation of spleen cells, human serum, and LCS were essential. The requirement for LPS varied with the donor. Insoluble antigen gave 3X and 15X higher IgM and IgG responses, respectively, than soluble antigen. In addition to BTT, this procedure has been used to procedure human MAb against other tumor-associated antigens isolated from human breast and lung tumor lines. Studies are in progress to examine the cellular subpopulations and growth factors required for antigen-specific activation of B lymphocytes in vitro.

A93 GROWTH FACTORS AS POTENTIATORS OF CYTOTOXIC DRUGS(1), Verena Hug, Dennis Johnston Margot Finders and Gabriel Hortobagyi, M.D. Anderson Hospital, Houston, TX 77030

We found that 17-β-estradiol, epidermal growth factor, hydrocortisone acetate, and insulin, alone and in combination, could increase the rate of proliferation of clonogenic breast tumor cells. This growth-stimulatory effect was observed for established breast tumor cells (MCF-7, MDA-468), and for fresh tumor cells. The magnitude of effect was proportional to the proliferative activity of cells, but was greater on the ER-positive MCF-7 cells and on cells from tumors of patients responding to endocrine treatment. Conversely, the tumor growth-inhibitory hormone, tamoxifen-citrate, inhibited the rate of proliferation primarily of the rapidly growing cell subpopulations, and the inhibitory effect was more pronounced on the estrogen receptor-positive cells.

The hormones changed the adriamycin-sensitivity of breast tumor cells. 17-β-estradiol increased it, and tamoxifen-citrate decreased it. The degree of change correlated with the proliferative activity of cells. Thus, 17-β-estradiol enhanced the adriamycin-sensitivity maximally of the slow-growing cell subpopulations, e.g. of the estrogen receptor-positive cells grown to confluence, in the case of established cells, and of the tumor cells from patients responding to hormone treatment, in the case of fresh tumor cells.

The effect of 17-β-estradiol and of tamoxifen-citrate was cell-type specific. Neither of the hormone did significantly influence the proliferative behavior and the adriamycin-sensitivity of GM-CFU.

(1)Cancer Research, in press.

A94 A CONSISTENT CHROMOSOMAL ABNORMALITY, 3p-, IN HUMAN SMALL CELL LUNG CARCINOMA LINES, Julia M. Ibson, Pamela H. Rabbitts, Jonathan J. Waters* and Peter R. Twentyman±, Ludwig Institute for Cancer Research, MRC Centre, Cambridge; *Department of Clinical Cytogenetics, Addenbrooke's Hospital, Cambridge; ±MRC Clinical Oncology and Radiotherapeutics Unit, Cambridge.

Twelve small cell lung carcinoma (SCLC) lines, 10 of which were derived from patients in Cambridge, have been classified on the basis of established morphological and biochemical criteria including levels of L-Dopa decarboxylase and creatine kinase BB (Baillie-Johnson et al., Br. J. Cancer, Oct. 1985). These lines were screened for amplification and expression of the c-myc and N-myc oncogenes. No consistent pattern of amplification of these genes with respect to the classic and variant subclasses of SCLC has emerged; 5 of 9 classic SCLC lines have N-myc amplification while 4 classic and 3 variant lines show no amplification of either oncogene. However, karyotypes of the lines show a striking correlation between the presence of a deletion of chromosome 3p and SCLC; 11 of the 12 SCLC lines have a 3p-. We have located the breakpoint more precisely than previously reported to 3p23-24. Interestingly, the one line lacking the 3p- has a translocation involving this same breakpoint, t(3;11) (p24;p15). We conclude that i) aberrations of the loci in the region of 3p23-24 are involved in the ontogeny of SCLC, ii) aberrations of 3p may be diagnostic of SCLC whereas the presence or absence of c-myc or N-myc amplification is not.

A95 ISOLATION OF TUMOR-ASSOCIATED ANTIGENS (TAA) FROM COLLOGENASE-DNASE DIGEST OF HUMAN COLORECTAL CARCINOMA (CRC). J. Milburn Jessup, Guevara, J., and Babcock, G.F. UT M. D. Anderson Hospital, Houston, TX, 77030.

Critical analysis of human host-tumor interactions requires the use of autologous tissues. Since such tissues are in short supply, supernatants of enzymatic dissociations of human CRC were utilized as a source of TAA. CRC and normal mucosa (NM) from operative specimens were dissociated with 0.3% Collagenase Type I and 0.03% DNase Type I at 37°C for 3-6 hr. The supernatants were collected and filtered, while the cells were used in an indirect membrane immunofluorescence (IMI) assay. When cells were incubated with a 1:8 dilution of serum, 15 of 21 sera reacted with autologous CRC while 4 of 21 reacted with autologous NM. Serum from an AB+ normal donor did not bind to CRC. When specificity was assessed against 6 CRC cell lines with 15 sera, little cross-reactivity was noted since 7 sera reacted with LoVo, 4 or fewer sera reacted with the remaining cell lines, whereas only 4 sera reacted with 2 or more cell lines. Antibodies from a patient that reacted with both autologous CRC and NM bound diffusely to cytoplasm of paraffin-fixed CRC and to the apical cytoplasm of columnar cells in upper third of mucosal crypts. IgG was isolated from the serum of this patient and used as an immunosorbent for CRC and NM digests. Substances in the NM digest were eluted from the immunosorbent and blocked the binding of autologous antibody to NM but not CRC in the IMI assay. Similarly recovered material from the CRC digest blocked binding of autologous IgG to both CRC and NM. This affinity-purified CRC TAA contained 8 peptides with pI 4.0-4.3 and molecular weight of 30-38 kDa. Thus, collagenase-DNase digests are a source of human CRC TAA that may be individually distinct.

A96 THREE DISTINCT FORMS OF PREPRO-GASTRIN RELEASING PEPTIDE (GRP) mRNA IN A SMALL CELL LUNG CARCINOMA (SCLC) CELL LINE. Anne-Marie Lebacq-Verheyden, Edward A. Sausville, Eliot R. Spindel* and James F. Battey. NCI-Navy Medical Oncology Branch, Naval Hospital, Bethesda MD 20814 and * Department of Medicine, Harvard Medical School, Boston MA

GRP is a neuropeptide widely distributed in the central and peripheral nervous system as well as in normal and transformed neuroendocrine cells. Recently, GRP was shown to act as a growth factor in vitro on normal bronchial epithelial cells (Willey et al, Exp.Cell Res. 153:245, 1984) and on SCLC cell lines (Weber et al, J.Clin.Invest. 75:306, 1985). The structure of 12 cDNA clones to human prepro-GRP mRNA derived from SCLC cell line NCI-H209 was examined. Three types of prepro-GRP mRNA were found. They differ in the structure of a putative GRP-associated peptide and predict the synthesis of 3 distinct pro-GRP molecules. Comparison of the sequence of cDNA clones with the sequence of a genomic prepro-GRP clone reveals that the 3 forms of prepro-GRP mRNA arise from a single primary transcript that undergoes alternative processing from 2 splice donor sites to 2 splice acceptor sites. S1 nuclease protection experiments demonstrated the presence of all 3 prepro-GRP mRNAs in SCLC line NCI-H209. The subcellular distribution of these transcripts and the structure of the prepro-GRP cDNA clones suggest that all 3 types could function as mRNAs. The biological function of the corresponding GRP-associated peptides remains to be elucidated.

A97 TUMORIGENICITY AND C-MYC AMPLIFICATION IN SEWA MOUSE TUMOR CELLS, Göran Levan, Tommy Martinsson and Fredrik Ståhl, Department of Genetics, Göteborg University, S-400 33 Göteborg, Sweden

Most sublines of the SEWA murine ascites tumor display the cytogenetic signs of gene amplification, i. e. double minute chromosomes (DM), homogeneously staining regions (HSR), or C-bandless chromosomes (CM; 1). These structures contain numerous copies of the c-myc oncogene (2). Regardless of the type of chromosome aberration present, the extra gene copies appear to be intact, since they yield restriction fragments of the same size as control cells, when digested with enzymes that cut well outside the coding sequences on both sides of the gene. In the SEWA subline Rec4TC1, amplified c-myc genes reside in CM. This line was subcloned and 5 clones were selected. Three of them had no CM at all, and 2 had levels of CM comparable to the parental line. Measurements of c-myc copy numbers indicated that the former had about 5-fold and the latter about 30-fold amplification compared to normal cells. Cells from the subclones were injected subcutaneously into 10-day old compatible mice. It was determined that there was a rough positive correlation between amplification of c-myc and tumorigenicity in this cell system.

(1) Levan, G. and Levan, A. In Gene Amplification (R. T. Schimke, Ed.), Cold Spring Harbor Laboratory, p. 91-97 (1982)
(2) Schwab, M., Ramsay, G., Alitalo, K., Varmus, H. E., Bishop, J. M., Martinsson, T., Levan, G. and Levan, A. Nature 315:345-347 (1985)

A98 PHENOTYPIC CHARACTERIZATION OF EWING SARCOMA CELL-LINES WITH MONOCLONAL ANTIBODIES
Marc Lipinski, Karim Braham, Irène Philip, Thierry Philip, Gilbert M. Lenoir and Thomas Tursz, Institut Gustave Roussy, Villejuif, France.

The histogenesis of Ewing sarcoma, the second most frequent bone tumor in humans, remains controversial. Nine Ewing cell-lines were analyzed by immunological methods. Surface antigens recognized on Ewing cells were found to be related to the neuroectoderm lineage. Ganglioside GD_2, a marker of neuroectodermal tumors, was present on all lines. All but one were also stained by the mouse monoclonal antibody HNK-1 that detects a carbohydrate epitope present on several glycoconjugates of the nervous system, including the myelin-associated glycoprotein MAG and the neural cell adhesion molecule N-CAM. Human monoclonal antibodies from patients with demyelinating neuropathy also reacting with MAG stained 5 of the 9 Ewing lines tested. The P61 rat antibody that reacts with a peptide moiety of N-CAM stained 7 lines. By contrast, all antibodies detecting cell surface antigens specifically associated with the hematopoietic lineage revealed totally unreactive. HLA class II antigens were never detected while the level of expression of class I antigens varied to a large extent. Ewing sarcoma cells are characterized by a t(11;22)(q24;q12) translocation that also occurs in neuroepithelioma, a neuroectodermal malignancy. Thus, Ewing sarcoma cells share a series of antigenic and karyotypic features with derivatives of the neuroectoderm that could indicate a similar histogenesis.

A99 Detection of Tumor Associated Antigen in Urine of Patients with Bladder Cancer by Using Fragments of Murine Monoclonal IgM.
Brian Liu, L. M. Stock, H. Neuwirth, and J. L. Fahey. UCLA School of Medicine, Los Angeles, CA 90024.

This lab has reported the isolation of two murine monoclonal IgM antibodies (E7 and G4) which were generated against human bladder cancer. We now report the use of E7 fragments to assay tumor antigen in urine.

E7 antibodies in nude mice ascitic fluid were purified by Sephacryl-300 MW column chromatography. Proteins were adjusted to 1mg/ml with sterile PBS, digested with trypsin, and reduced with mercaptoethanol. SDS gel electrophoresis of digested immunoglobulins reveal fragments with MW between 100 and 250 kdaltons.

Normal adult urines and urines from patients with known transitional cell carcinoma (TCC) metastasis were obtained and processed with centrifugal microconcentrators. Using human bladder cancer cell line 647V as a reference antigen linked to a solid support, whole or fragmented E7 was mixed with the test urine, and the mixture was then incubated with the solid phase, which was then washed. Peroxidase conjugated rabbit-anti-mouse immunoglobulin was then added, followed by enzyme substrate. Preliminary results indicate that urine samples from patients with TCC were able to prevent the binding of fragmented but not intact E7 to the reference antigen. Normal adult urines and urines from patients with non-TCC bladder cancers did not inhibit the binding of fragmented E7. This suggests that fragmented, but not pentameric E7 will be useful for sensitive inhibition assays for antigens in urine of patients with TCC.

A100 EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) IN HUMAN PANCREATIC CARCINOMA: RELATION TO NUMERIC AND STRUCTURAL ALTERATIONS OF CHROMOSOME 7.
P. Meltzer, J. Trent, M. Korc, Depts of Pediatrics, Radiation Oncology & Internal Med, Univ. of AZ Health Sciences Center, Tucson, AZ 85724

Recently, it has been proposed that levels of EGFR expression in human melanoma correlate directly with the number of copies of chromosome 7 (the locus of the EGFR gene), but not with structural alterations of this chromosome (Koprowsky et al., Somat Cell Mol Genet 11:297,1985). In contrast, structural alterations of chromosome 7 (often associated with amplification of EGFR sequences) have been described as the major mechanism of EGF overexpression in squamous cell carcinomas. In this study, we report the analysis of three human pancreatic carcinoma cell lines (Panc-I, T_3M_4, and UACC/462) for ^{125}I-EGF binding analysis, EGF gene copy number, mRNA expression, as well as numeric or structural alterations of chromosome 7. Saturation analysis revealed high numbers of EGFR on two of the three cell lines ($4x10^5$-Panc I; $1.2x10^6$-T_3M_4) and moderately elevated EGFR levels on the third ($6x10^4$ - UACC/462). DNA analysis failed to demonstrate an increase in EGFR copy number in any of the three cell lines. Both of the EGF overproducing lines displayed clonal structural alterations resulting in over-representation of the short arm of chromosome 7. The single cell line not exhibiting a marked overabundance of EGFR (UACC/462) had no structural alteration of chromosome 7 but did have an increased number of copies of a normal chromosome 7. Our results suggest that EGFR overexpression in pancreatic carcinoma can be found in conjunction with either structural or numeric alterations in chromosome 7.

A101 MONOCLONAL ANTIBODIES TO THE EGF RECEPTOR SELECTIVELY INHIBIT THE GROWTH OF HUMAN TUMOR CELLS. J. Mendelsohn, H. Masui, H. Sunada and C. MacLeod. Memorial Sloan-Kettering Cancer Center, New York, NY.

EGF stimulates the proliferation of fibroblasts and most epithelial cell types, whereas it profoundly inhibits the growth of A431 epidermoid carcinoma cells. The growth of 8 EGF receptor-bearing human tumor cell lines was measured following the addition of EGF or monoclonal anti-EGF receptor antibody 528 IgG2a (which blocks EGF binding). Epidermoid carcinoma cell lines from lung (T222), skin (T423), and vulva (A431) were growth inhibited by both EGF and 528 IgG. Proliferation of the other 5 human tumor cell lines tested was not blocked by either EGF or 528 IgG. Xenografts of the 3 cell lines inhibited by EGF and 528 IgG in culture were inhibited by 528 IgG treatment in vivo, whereas the other 5 tumors were unaffected. Differences in the number of EGF receptors expressed on the cell surface did not account for the inhibition of selected receptor-bearing tumor cells. Monoclonal antibody 225 IgG1 also prevented proliferation of A431 cells in culture and in xenografts. Screening for complement-mediated and cellular mechanisms of cell kill demonstrated cytolytic effects of macrophages upon A431 cells in the presence of 528 IgG2a, but we could find no immune mechanism to explain the anti-tumor effect on 225 IgG1. Thus, the anti-proliferative activity may be related to direct effects upon the receptor. In summary, immunotherapy of xenografts with anti-EGF receptor antibody is effective against a subset of receptor-bearing cells, which are also, in all cases, inhibited in vitro. NIH CA-33397. *C. MacLeod is at U.C. San Diego, La Jolla, CA.

A102 PRIMARY CHROMOSOME CHANGES IN NEOPLASIA, F. Mitelman and S. Heim, Department of Clinical Genetics, University Hospital, S-221 85 Lund, Sweden

In an attempt to identify chromosomal aberrations of prime significance, the data included in the Catalog of Chromosome Aberrations in Cancer (1985) was surveyed to ascertain all neoplasms having one single numerical or structural chromosomal abnormality as the only deviation from the normal karyotype.

A numerical aberration was present as the sole cytogenetic change in 610 of the 5 345 cases (11.4%). The distribution of gains and losses was clearly nonrandom with preferential gain of chromosomes 8, 9, 12, and 21; loss of chromosomes 7, 22, and Y.

318 different types of structural aberrations were found as the sole abnormality; 77 were identical in at least two neoplasms, and the 161 breakpoints in these aberration types were restricted to a total of 83 bands. At least one of the 83 bands was involved in 97% of all neoplasms where each structural rearrangement had been exactly identified.

The results indicate that genes important in tumor development may be located in a restricted number of chromosomal regions. A comparison between these cancer-associated breakpoints and the distribution of nonrandom, simple numerical aberrations should help further define the interaction of genes involved in the neoplastic process.

A103 HIGH AFFINITY VIP RECEPTORS ARE PRESENT ON SCLC CELLS. T.W. Moody[1], M.M. Shaffer[1], L.Y. Korman[2], D.N. Carney[3], Z-C. Zhou[4], and R.T. Jensen[4]. [1] Dept. Biochemistry, GWU Medical Center and [2]Gastroenterology Section, VA Medical Center, Washington, D.C., [3]Mater Hospital, Dublin, Ireland and [4]Digestive Disease Branch, NIDKD, Bethesda, MD.

Numerous neuropeptides have been shown to modulate growth. Among these include bombesin, which functions as an autocrine growth factor in small cell lung cancer (SCLC) cells (Cuttitta et al., Nature 316: 823, 1985) and vasoactive intestinal peptide (VIP) and stubstance P which modulate growth of immune cells (Ottaway and Greenberg, J. Immun. 132: 147, 1984). Recently, we have shown that VIP stimulates adenyate cyclase activity in SCLC cells (Korman et al., Cancer Res., in press) resulting in increased secretion of bombesin-like peptides. This prompted the search for VIP receptors in SCLC cells.

Here we report that ^{125}I-VIP binds with high affinity (Kd = 4 nM) to a single class of binding sites (Bmax = 4,000/cell using cell line NCI-N592). Pharmacology studies indicated that peptides structurally similar to VIP, such as GHRH, secretin and PHI, inhibited binding with high affinity whereas peptides structurally unrelated such as bombesin and substance P did not. Biochemical studies indicated that ^{125}I-VIP was cross linked to a protein of 45,000 daltons using disuccinimylsuberate (1 mM). These protein receptors for VIP may modulate the growth of SCLC cells.

A104 Karyotypic analysis of Harvey ras transformed rat embryo cells, their tumors and metastases reveals essentially diploid cells by Ruth J. Muschel, Ken Nakahara, Elizabeth Chu, Rudy Pozzatti, George Khoury and Lance Liotta. National Cancer Institute, Bethesda MD

Recently, a number of investigators have succeeded in transforming early passage rodent cells with the Harvey ras oncogene. Pozzatti et al (manuscript submitted) were able to transform early passage rat embryo cells using the Harvey ras oncogene. Every clone tested was metastatic in both lung metastasis and spontaneous metastasis assays. Because the early passage fibroblasts are diploid and yet are metastatic after transformation, we elected to study the karytopyes of these cells. Of two transformed clones analyzed, one was diploid, the other had a trisomy of chromosome 4. No banding abnormalities were seen in either. Cells were established from the tumors, lung nodules and metastases which resulted from the injection of these cells in nude mice. In no case did the tumors or metastases differ in karyotype from the cells prior to injection. Since most tumors and metastatic cell lines are reported to be highly aneuploid, it has not been possible to look in these systems for the selection of karyotypic abnormalities specific to metastasis. Our analysis of the karyotypes of the transformed rat embryo cells and of the tumors and metastases derived from them indicate that in this system karyotypic alterations are probably not required for metastatic behavior.

A105 BESTATIN FOR INDUCTION OF CANCER REDIFFERENTIATION, Shinichi Okuyama and Hitoshi Mishina, Departmnet of Radiology, Tohoku Rosai Hospital, Sendai 980, Japan

Bestatin decreases saturation density of mammalian cell culture (Tohoku J. Exp. Med. 142, 349, 1984) and induces morphological redifferentiation in vitro, especially in combination with sex hormones when FM3A undifferentiated mammary adenocarcinoma cells of the mouse were employed (Ibid., 143, 501, 1984). Maturation of the cellular microvilli and processes, and their eventual degeneration were observed by scanning electron microscopy. The principle was applied to those patients with cancer of the breast or prostate. Observed were return of sensitivity to the once-resistant hormones and return of the contact inhibition as the cells acquired nucleosomal enlargement and ceased to pile up as monitored by a serial histopathology (Ann. New York Acad. Sci., in press). The lymphocytic infiltrations disappeared, too. Inhibition of metastases was also suspected. Retrieval of cellular polarity was observable with cases of the stomach cancer and so on. Bestatin reduces cellular membrane negative charge when it binds to hydrolytic enzymes of leucine aminopeptidase and aminopeptidase beta on cell surfaces, thus triggering redifferentiation of cancer cells and leading to eventual cancer cell loss (J. Clin. Hematol. Oncol. 15, 43, 1985). Bestatin may thus help cultivate a new principium of cancer therapy.

A106 MAMMALIAN CELL TRANSFORMATION BY A MURINE RETROVIRUS VECTOR CONTAINING THE AVIAN ERYTHROBLASTOSIS VIRUS erbB GENE, J. Pierce, A. Gazit, C. Pennington, M. Kraus, P. DiFiore, S. Aaronson, National Cancer Institute, Bethesda, Maryland 20892

A recombinant murine retrovirus vector containing the v-erbB gene of avian erythroblastosis virus, designated MuLV/erbB, was constructed in order to investigate the function of v-erbB as a transforming or growth-promoting gene in mammalian cells. NIH/3T3 cells transfected with MuLV/erbB DNA induced transformed foci at a high efficiency. Transmissible MuLV/erbB retrovirus was generated by infection of nonproducer foci with amphotrophic murine leukemia virus (A. Gazit et al., manuscript in preparation). MuLV/erbB-induced foci had a dense fusiform morphology. Individual MuLV/erbB transfectants grew in soft agar and were tumorigenic. These transfectants were also shown to contain v-erbB DNA, to express multiple v-erbB-specific transcripts, and to synthesize v-erbB-related glycoproteins. Most transfectants produced two major v-erbB gene products of 70 and 68 kilodaltons. However, some transfectants produced smaller v-erbB-specific proteins. The size heterogeneity observed between different transfectants was not the result of variation in glycoprotein processing. Therefore, it is likely that alterations in the MuLV/erbB genome may have occurred during the transfection process and in some cases led to the creation of v-erbB transforming mutants coding for smaller molecular weight products. We have introduced the v-erbB gene via the mammalian retrovirus vector into a variety of mammalian cell culture systems and in vivo. The spectrum of cells whose growth and differentiation can be altered by this activated homologue of the EGF receptor gene will be discussed.

A107 IMMUNOHISTOCHEMICAL AND IMMUNOCYTOCHEMICAL DETECTION OF LUNG CARCINOMA WITH MOUSE MONOCLONAL ANTIBODIES. R. Ranken, T. Gottfried, R. Jones, V. Liu, P. Nelson, and C. White, Carcinex, Burlingame, CA 94010.

Mouse monoclonal antibodies were derived from the fusions of mouse spleen cells which were immunized with squamous cell tumors and adenocarcinoma tumors. The antibodies were extensively screened by immunofluorescence on frozen sections of different tumor types as well as tissues of squamous cell and non-squamous cell origin. The degree of tumor association with normal components has been assessed.

The utility of these antibodies in the immunohistochemical and immunocytochemical detection of the four types of lung cancer is illustrated. Nitrocellulose and polystyrene bead solid phase immune assay has demonstrated the feasilbility of detecting antigen in lung washings and sputum. The antibodies are also being evaluated for early imaging of lung lesions. Biochemical characterization is in progress as well as the generation of preliminary data for their use in the clinical setting.

A108 A BIOLOGICAL ASSAY TO PREDICT FOR THE HORMONAL RESPONSIVENESS OF BREAST TUMORS, Jung Ro, Verena Hug and Gabriel Hortobagyi, M.D. Anderson Hospital, Houston, TX 77030

We determined the in vitro clonogenicity of tumors under regular and under hormone enriched (17-β-estradiol, epidermal growth factor, insulin, hydrocortisone) culture conditions, and took the ratio of colony-formation under the 2 conditions as the measure to predict the in vivo hormonal responsiveness of tumors. We assayed the tumors of 19 pts who subsequent to the test received hormonal treatments (tamoxifen 8, megace 3, aminoglutethimide 3, others 5). Seven pts responded to the treatments (2 CR's, 5 PR's), and 11 pts failed to respond (1 stable and 10 progressed). One pt was too early to assess for response.

All 19 tumors were considered to be hormonally dependent, but the estrogen receptor was known in only 11. It ranged from 7-272 fm/mg cytosol protein, using the dextran coated charcoal method of determination. 6 of the ER-positive pts responded, and 5 failed.

The in vitro hormonal responsiveness of tumors was calculated as follows:

$$\log_{10} \frac{\text{number of colonies with hormones}}{\text{number of colonies without hormones}}$$

This ratio ranged from -0.19 to +1.58. The median ratio for responders was +0.49, and that for failures +0.16.

We used +0.30 as the cut off point to discriminate between hormone responders and hormone failures. With this cut off point, the test identified all responders (100% sensitivity), and its positive predictive value was 78% and it negative predictive value 100%.

A109 A GROWTH ASSOCIATED PROTEIN REGULATED BY ESTROGENS AND SECRETED BY HUMAN MAMMARY CANCER CELLS, Henri Rochefort, Françoise Capony, Marcel Garcia, Muriel Morisset and Françoise Vignon, Unité d'Endocrinologie Cellulaire et Moléculaire (U 148) INSERM, 60 Rue de Navacelles, 34100 Montpellier France.

The study of estrogen regulated proteins may be valuable in understanding the mechanism by which estrogens stimulate cell proliferation and mammary carcinogenesis. In estrogen receptor positive human breast cancer cell lines (MCF7, ZR75-1) estrogens specifically increase the production into the culture medium of a 52,000 dalton (52 K) protein (Cell 20: 352-362, 1980) and stimulate cell growth. The antiestrogen do not themselves induce the 52 K protein, but inhibit its induction by estrogens.

Using monoclonal antibodies and indirect immunoperoxydase staining of frozen sections, the 52 K protein has been detected in epithelial cells of some breast cancers and benign mastopathia but not in normal mammary gland and other estrogen target tissues. By immunoaffinity, the 52 K protein has been purified to homogeneity in the medium and in the cell extract. The protein is N-glycosylated with high mannose oligosaccharide chains which are phosphorylated in vivo on mannose. The secreted 52 K protein can be uptaken by MCF7 cells and processed into 48 K and 34 K proteins which accumulate in lysosomes. This uptake and processing are specifically inhibited by Man-6-P and lysosomotropic agents suggesting that the 52 K protein is targeted to lysosomes via Mannose 6-P receptors.

In vitro the purified 52 K protein stimulates the growth of MCF7 cells and is associated to a protein kinase activity. The 52 K protein may therefore be an autocrine or paracrine mitogen mediating the effect of estrogens on the growth of mammary cells.

A110 AUTOSTIMULATORY PRODUCTION OF A HEMOPOIETIC GROWTH FACTOR IN MYELOID LEUKEMIA. John W. Schrader, Kevin Leslie, Sabariah Schrader and Hermann J. Ziltener, The Walter and Eliza Hall Institute of Medical Research, Parkville 3050, Australia.

Three murine myeloid leukemias have been shown to constitutively produce a hemopoietic growth factor, panspecific hemopoietin (PSH) or IL-3, that is normally produced by activated T lymphocytes. Two of these, WEHI-3B and WEHI-274.14 arose in vivo and were cloned from mice exposed to mineral oil or Abelson murine leukemia virus, respectively. The constitutive production of PSH appears to result from aberrant activation of the PSH gene; in both WEHI-3B and WEHI-274.14 one PSH gene is rearranged and in the case of WEHI-274.14 the major RNA transcript is much larger than normal. WEHI-274.14 shows an autostimulatory pattern of growth in vitro; at low but not high cell-densities growth is strongly dependent on the external concentration of PSH. A third series of leukemic lines were derived in vitro from a non-leukemogenic, PSH-dependent mast cell/megakarocyte line as spontaneous variants able to grow in the absence of exogenous PSH. These variants produced a factor with the biological and biochemical properties of PSH and unlike the parental line contained PSH mRNA. In vitro growth was strongly dependent on cell density and the external concentration of PSH Moreover, the onset of autogenous PSH production coincided with the onset of leukemogenic behaviour. Binding studies with antibodies to synthetic peptides showed that PSH molecules from the three leukemic lines had the same N- and C-termini as PSH from T-cells. Antibodies directed towards synthetic PSH together with synthetic analogues of PSH are being tested for anti-leukemia activity in vitro and in vivo.

A111 GENES FOR TUMOR MARKERS ARE CLUSTERED NEAR CELLULAR ONCOGENES, Jill Siegfried, Marc Mass and John Hozier, Environmental Health Research and Testing, Inc., RTP, NC, Environmental Protection Agency, RTP, NC, Florida Institute of Technology, Melbourne, FL.

The aberrant expression of normal mammalian genes in association with malignant transformation (tumor marker genes) has been a matter of intensive study for many years, primarily in attempts to reconcile such abnormal gene expression with development of the malignant phenotype. More recently, the discovery of mammalian genes homologous to retroviral oncogenes (the cellular proto-oncogenes) has led to the hypothesis that activation of such cellular oncogenes is causally related to malignant transformation. We have analyzed the relative mapping positions of genes for tumor marker proteins and cellular proto-oncogenes and find a remarkable degree of co-mapping of tumor markers with oncogenes in the human karyotype. Of thirty-one tumor markers for which there is documented evidence of abnormal expression in tumors and a sufficient degree of chromosomal sub-localization (to within 5% of the human genome), twenty-seven map to chromosome segments overlapping or adjacent to cellular oncogenes. An analysis of the probability that such co-mapping is due to chance shows instead a highly significant clustering of tumor marker genes in the proximity of cellular oncogenes. We propose that aberrant expression of marker genes in tumors may be directly related to their proximity in the human genome to specific proto-oncogenes expressed during the development of malignancy, and we suggest ways in which this hypothesis of concerted abnormal gene expression in mammalian tumor cells may be tested.

A112 RECURRENT CHROMOSOME REARRANGEMENTS IN MALIGNANT AND BENIGN ADIPOSE TISSUE TUMORS, Claude Turc-Carel, Janusz Limon, Paola Dal Cin and Avery A. Sandberg, Roswell Park Memorial Institute, Buffalo, NY 14263

An analysis of chromosome aberrations was performed in 7 consecutive cases of adipose tissue tumors: one benign lipoma and 6 malignant liposarcomas classified as myxoid (4 cases), poorly differentiated (1 case) and well differentiated (1 case). Patients ages ranged from 36 to 75 years. Specimens studied were from primary untreated tumors in 3 cases and from recurrences after treatment in 4 cases. The studies were performed on collagenase disaggregated tumor cells cultured for 2 to 10 days. Modal chromosomal number ranged from 46 to 85. Clonal numerical and/or structural abnormalities were observed in G-banded metaphases of every case. Chromosome #12 was consistently involved, including 3 cases with translocations with identical breakpoints at 12q13 and 16p11, all 3 being myxoid liposarcomas. The benign lipoma cells displayed a translocation involving chromosome #12 at band 12q14 and chromosome #3 at band q28. These translocations were considered primary karyotypic changes.

Recurring abnormalities involving the long arm of chromosome #12 in human neoplasia are a rather rare event. The possible role played by the oncogene c-int 1 and a fragile site at 12q13 in these tumors is being investigated. The clinical significance of these chromosome changes in malignant and benign adipose tissue tumors will be discussed.

A113 CHARACTERIZATION OF A GENE CODED ON CHROMOSOME 13 WHICH IS KNOWN TO BE LINKED TO RETINOBLASTOMA, Jeremy A. Squire, Hunt Willard, Maria A. Musarella, Theodore Hoffman, Brenda L. Gallie, and Robert A. Phillips, Ontario Cancer Institute, Toronto, Canada M4X 1K9.

Esterase D is known to be closely linked to the locus responsible for retinoblastoma on chromosome 13. In order to isolate this gene for use as a DNA probe for molecular investigations of retinoblastoma and chromosomal walking experiments, we have carried out the following cloning strategy. An esterase D protein of 30,000 daltons was purified to homogeneity and a partial amino acid sequence was obtained. Two different mixed oligonucleotide probes based on this sequence were synthesized and used to screen a human cDNA library. Positive clones independently recognized by both oligonucleotides have been characterized as a single gene, coding for a polypeptide of the correct size. On Northern blots, the probe identifies a single 1.8Kb message in all cells examined. This gene has been mapped to chromosome 13 by screening a human-rodent hybrid panel; it also identified the same restriction fragments in a sorted chromosome 13 library.

A114 EXPRESSION AND ACTIVITIES OF EPIDERMAL GROWTH FACTOR RECEPTOR AND ASSOCIATED GLYCOPROTEIN ON CULTURED HUMAN GLIOMA CELLS. Peter A. Steck, Gary E. Gallick, Steve A. Maxwell, and W. K. Alfred Yung, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030

The aberrant expression of epidermal growth factor receptor (EGF-R) has been recently observed in some primary human brain tumors. We have also identified a closely associated glycoprotein (M_r~190,000; p190) and EGF-R on cultured glioma cells by several independent techniques. Examination of these two glycoproteins has been performed to ascertain biochemically the similarities and differences between p190 and EGF-R. Phosphotyrosine was a major phosphorylated residue demonstrated from both proteins after phosphorylation assays of functional immunoprecipitated EGF-R-complex-kinase or membrane preparations. A kinetics pulse-chase experiment revealed that these two proteins did not represent precursor products of each other; p190 exhibited a longer half life (>6 h) than EGF-R(~4 h). The two proteins generated relatively similar, but distinct tryptic polypeptide fragments as determined by Cleveland analysis. Preliminary glycoconjugate analysis suggests p190 does not represent an over-glycosylated form of EGF-R. Furthermore, the protein kinase activity of EGF-R was stimulated by exogenously added epidermal growth factor, but p190 was not. These results suggest a close similarity of p190 and EGF-R, expressed by cultured human glioma cells. The possible relationship between p190 and neu oncogene protein product (M_r~185,000) is being investigated.

A115 CHARACTERIZATION OF HIGH MOLECULAR WEIGHT TRANSFORMING GROWTH FACTORS (HMW TGFs) IN CONDITIONED MEDIUM OF HUMAN RHABDOMYOSARCOMA CELL LINE A673, Kurt Stromberg[1], W. Robert Hudgins[1], Charlotte M. Fryling[1], John R. Dedman[2], Robert L. Pardue[2], and William R. Hargreaves[3], [1]NCI-FCRF, Frederick, MD 21701, [2]Triton Biosciences, Inc., Houston, TX 77030, [3]Biotope, Inc., Bellevue, WA 98009

Extracts of serum-free conditioned medium from A673 cells contain HMW TGF(s) after partial purification by Bio-Gel P-100 and carboxymethyl cellulose chromatography (Todaro, G.J., Fryling, C.M., and De Larco, J.E., PNAS 77: 5258, 1980). Further characterization with reverse phase HPLC revealed a principal peak of coincident EGF receptor competition and soft agar activity at 25-26% acetonitrile. If a side shoulder of EGF radioreceptor activity from the CM-C chromatography was also included for further analysis, additional active HPLC fractions were observed at 22-23% acetonitrile. Importantly, both active elution regions from HPLC failed to compete in radioimmunoassays under reduced and denatured conditions for hEGF (D.N. Orth, Vanderbilt Univ.), or rat TGFα, and failed to give positive signals in Western blots under conditions in which rTGFα was readily detected when using an antisera raised against the 17 C-terminal amino acids of rTGFα. Non-reducing SDS-PAGE of the HMW TGFs from HPLC revealed EGF radioreceptor and soft agar activity in gel slices corresponding to Mr 15,000, 22,000 and 50,000 in the HPLC sample eluting at 25-26% acetonitrile, and an additional Mr 27,000 species in the HPLC sample eluting at 22-23% acetonitrile.

These multiple forms of human HMW TGF produced in vitro suggest size heterogeneity and possible immunologic diversity in the EGF/TGFα family of growth-promoting polypeptides.

A116 MOLECULAR GENETIC DIFFERENTIATION OF TWO HISTOPATHOLOGICALLY INDISTINGUISHABLE PEDIATRIC TUMORS; NEUROEPITHELIOMA AND NEUROBLASTOMA

Carol J Thiele, Timothy Triche+, Robert Ross* and Mark A Israel, Pediatric and Pathology+ Branch,COF,NCI,NIH;and Fordham University*, New York.

Adult neuroblastoma or neuroepithelioma (NE) is a peripheral nervous system tumor which is histopathologically indistinguishable from advanced stage, childhood neuroblastoma (NB). Cinically, NE presents on the trunk or extremities of older children and in this regard is unlike NB which usually occurs in children under two years of age in the adrenal gland or known sympathetic ganglia. The N-myc oncogene is amplified in approximately 50% of all Stage III and IV NB. Virtually all NB cell lines have amplified N-myc DNA and express high levels of N-myc mRNA. Utilizing Southern and Northern blot analysis, we have analyzed six NE cell lines and several tumor specimens and found that unlike NB cell lines, NE contain a single copy of the N-myc gene per haploid genome and express relatively low levels of N-myc mRNA. NE cell lines and tumors however contain high mRNA levels of the structurally and functionally related c-myc oncogene compared to NB. Analysis of NE and NB cell lines for neurotransmitter enzymes revealed that NE had high levels of cholinergic enzymes while NB expressed enzymes characteristic of adrenergic neurons. These molecular genetic and biochemical differences support our contention that these tumors are not different clinical presentations of the same tumor but in fact are distinct tumor entities.

A117 ALTERED DNA METHYLATION PATTERNS WITHIN THE MOUSE L1 AND HUMAN KPN FAMILIES OF ONCOGENICALLY TRANSFORMED TISSUES AND CELL LINES

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We are studying the possibility that stable alterations in the methylation patterns of repeated sequences may provide one type of clonal marker in neoplastic cells. Using pFS-13, a cloned mouse repetitive DNA sequence, as a probe in southern blots, mouse spleen and L1210 Lymphoma DNA appeared to be normally modified at Hpa II sites. Friend Erythroleukemia cell, and Sp2 cell DNA both showed the same characteristic banding pattern in Hpa II digests, indicating deranged methylation. The DNA sequence of the 1335 bp insert in the clone was determined; a copy of the "R" sequence of L1 was found at its Bam end. Walking experiments using M13 subclones from pFS-13 permitted the construction of a map for unmethylated 5'CCGG sites. The Hpa II pattern in Friend cells could then be assigned to subgroups within L1, homologous to BAM5 and R. Using an homologous KPN family clone, we found discrete banding patterns indicating concerted differences in KPN methylation in HpaII digested DNA from human lung tumors, and HeLa cells. Low levels of methylation seen in lymphocyte DNA from patients with chronic lymphocytic leukemia do not appear to extend to DNA from the KPN family.

A118 A GLYCOLIPID ANTIGEN ASSOCIATED WITH BURKITT'S LYMPHOMA, Thomas Tursz, Joëlle Wiels, Marc Lipinski, Eric Holmes*, Nancy Cochran*and Sen-itiroh Hakomori* , Institut Gustave Roussy, 94805 Villejuif -France and Fred Hutchinson Cancer Research Center* Seattle, USA.

We have previously described the monoclonal antibody, 38.13, reacting with most Burkitt's lymphoma derived lines, either containing the Epstein-Barr virus (EBV) or not, but not reacting with EVB-positive lymphoplastoid cell lines. The target antigen, BLA, was shown to be a neutral glycolipid, identified as globotriaosylceramide (Gal α1- 4 Gal β 1-4 Glc β 1-1 ceramide) (Science 220 : 509, 1983). This substance is known as the blood group antigen Pk, a normal intermediate in the P substance synthesis. The enzyme activities involved in the synthesis and degradation of globotriaosylceramide, and the degree of exposure of this glycolipid have been studied with various Burkitt and non-Burkitt lymphomas and lymphoblastoid cell lines. The activity of UDP-Gal : LacCer α -galactosyltransferase of Burkitt lymphoma cells was consistently higher than that of non-Burkitt lymphoblastoid cell lines, and the enzyme activity can be correlated with the antigen expression at the cell surface. Though not detected on normal B cells from peripheral blood, bone marrow and lymph nodes, BLA was found on a small subset of tonsil B cells. BLA could behave as a "homing" antigen, with possible implications for the peculiar clinical localization of Burkitt's lymphoma.

A119 PROLIFERATION AND IDIOTYPE SECRETION BY HUMAN B CELL LYMPHOMAS IN VITRO. Florry A. Vyth-Dreese and Annemarie Hekman. The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Tumor cells of 5 human B-cell non-Hodgkin's lymphomas were studied for their capacity to proliferate and differentiate in vitro. B-cell populations obtained from the peripheral blood, pleural fluid, lymph node or spleen were cultured in serumfree medium for up to 9 days and tested for ^{3}H-thymidine incorporation and secretion of idiotypic immunoglobulin (Id) into the culture fluid. Before and after culture the surface marker profile of the cell populations was determined as well. The results indicate that neoplastic B-cell populations can "spontaneously" proliferate in culture depending on their state of activation in vivo. The highest proliferative responses were found with cell populations positive for 4F2 antigen and co-expressing Tac antigen or acquiring it during culture. These cell populations also responded directly to B-cell growth factor (a gift of Dr. Fauci). Id secretion was observed in proliferating as well as non-proliferating cell cultures. The Id detected could not be attributed to leakage from dead cells and was of IgM isotype. Furthermore, a correlation was found between the amount of Id secreted in vitro and the serum Id level of the lymphoma patients. Therefore, these results are of use for the strategy of lymphoma therapy with anti-Id monoclonal antibodies.

A120 OUABAIN-INDUCED DIFFERENTIATION OF EMBRYONAL CARCINOMA, Barbara T. Zimmerman and Wendell C. Speers, Department of Pathology, University of Colorado Health Sciences Center, Denver, CO 80262.

The murine embryonal carcinoma (EC) cell line PCC4-azal can be induced to undergo morphologic differentiation by a variety of chemical agents including retinoic acid and dimethyl acetamide. However, the mechanisms by which these agents exert their effects are not well understood. We have observed that ouabain (G. Strophanthin) also induces differentiation in these cells at concentrations which inhibit Na^{+},K^{+}-ATPase activity as measured by suppression of $^{86}RB^{+}$ uptake. Since the Na^{+},K^{+}-ATPase maintains ionic gradients in cells, we investigated the possibility that alterations of ion flux and concentrations may signal the cascade of events resulting in morphologic differentiation. Results of numerous studies utilizing ionophores, channel blockers, and media deficient in specific components, failed to demonstrate a consistent role for ion flux in the differentiation process. We are currently investigating the possibility that altered ATP utilization may be the critical inducing stimulus. We are also attempting to rule out the possibility that ouabain action in inducing EC differentiation is an indirect phenomenon that is not mediated by the Na^{+},K^{+}-ATPase. Support: Grants CA 09157, CA 15823 and CA 33800 from the NIH and a departmental gift from R.J. Reynolds Industries, Inc.

Tumor Heterogeneity, Progression and Metastasis

A121 THE USE OF BASEMENT MEMBRANE MATRIX GELS FOR STUDIES ON TUMOR CELL 'HAPTOINVASION' A.Albini, Y. Iwamoto, J.O.Graf, G.T.Kitten, H.K.Kleinman, S.Aaronson*, A.Veillette*, M.E.Lippman* and G.R.Martin, LDBA, NIDR and *NCI, NIH, Bethesda, MD 20892.

Tumor cells must invade basement membranes in order to metastasize. We have used a reconstituted three dimensional basement membrane gel to measure tumor cell invasion in vitro or 'haptoinvasion'. The gel was overlaid on either culture dishes or chemotaxis filters in Boyden chambers. Cells which invaded and migrated through the gel and/or formed colonies inside it were quantitated and/or subcultured. Malignant cells such as PA-1 teratocarcinoma, SW620 colon adenocarcinoma, A-204 rhabdomyosarcoma, PC-3 prostatic carcinoma, Malme-3M melanoma, ME-180 cervix carcinoma and B16-Br2P mouse melanoma cells; low malignant cells such as HT-1080 fibrosarcoma, MCF7 breast carcinoma and ras-transfected NIH 3T3 cells; and non-tumorigenic cells such as $10t^{1}/_{2}$ and NIH 3T3 fibroblasts were tested. Malignant cells had the highest penetration into and growth inside the gel, and were the most migratory in response to laminin and to fibroblast-conditioned medium in the Boyden chamber assay (10-50 cells/high powered field). Low metastatic cells showed some migration (1-5 cells/field) and non-tumorigenic cells showed virtually no migration. The Br2P cells which had migrated through the gel were found to migrate twice as much as the parent cell population. In addition, MCF7 cells migrated through the coated filters and proliferated in the matrix gel to a greater extent if pretreated with estradiol or if transfected with the v-ras^{H} oncogene than MCF7 cells cultured in estradiol-depleted serum. The use of a biologically relevant material such as the basement membrane gel (Collaborative Research) in the haptoinvasion assay may be useful in quantitating the invasiveness of tumor cells and selecting highly invasive cell populations.

A122 EVALUATION OF PREMALIGNANT NEVI AS PRECURSORS OF METASTATIC MOUSE MELANOMAS, Jane Berkelhammer, AMC Cancer Research Center, Denver, CO 80214

The present study was designed to investigate the role of melanotic nevi in the pathogenesis of malignant mouse melanomas. We have recently confirmed that carcinogen-treated C57BL/6 mice develop a variety of melanotic and amelanotic skin lesions: 10% or less develop primarily melanotic tumors; approximately 30% develop progressively growing tumors with both melanotic and amelanotic areas; however, greater than 90% of the treated mice develop black nevi similar to human nevi. To evaluate the tumorigenicity of these nevi, we transplanted nevi from 35 carcinogen-treated mice to individual athymic nude mice. Of the first 16 nude mice evaluated, 10 have developed progressively growing tumors at the site of implantation. Seven of the tumors arose from nevi derived from tumor-bearing mice, and 3 tumors developed from nevi derived from mice with no apparent malignant tumors. Thus, 60% of the transplanted nevi exhibited "malignant potential" even though they did not exhibit progressive growth in the autochthonous host. Those nevi that invaded through the dermis in situ appeared to grow more readily after transplantation to nude hosts than did nevi confined to the epidermis. Our results suggest that the melanotic nevi may be precursors of malignant lesions or occult primary melanomas similar to those that develop from nevi in man. A thorough investigation of these premalignant lesions will provide valuable information about the development and progression of malignant melanomas in a clinically relevant experimental model. Supported by grant CA-38110 from NIH and a gift to the AMC Cancer Research Center from the McDonnell Douglas Foundation.

A123 AMPLIFICATION OF THE pVCR5L-18 GENE IN MULTIDRUG-RESISTANT CHINESE HAMSTER CELLS. June L. Biedler, Tien-ding Chang, Peter W. Melera, Kathleen V. Scotto and Barbara A. Spengler, Memorial Sloan-Kettering Cancer Center and The Rockefeller University New York, NY 10021

Recent studies have demonstrated that amplification of one or more genes is a prevalent and possibly requisite factor in acquired multidrug resistance. A group of Chinese hamster cell lines was probed with a cloned cDNA (pVCR5L-18) isolated from vincristine (VCR)-resistant DC-3F/VCRd-5L cells (2750x resistant) to determine chromosomal location of single copy and amplified pVCR5L-18 genes. Six cell lines were selected in vitro with actinomycin D (AD), daunorubicin (DM), or VCR from Chinese hamster lung DC-3F or bone marrow CLM-7 cells. Southern analysis indicated that amplification levels are 2- and 4-fold in the 2450x DC-3F/AD X and 50,000x resistant DC-3F/AD XC cells, respectively, 30-fold in DC-3F/DM XX (883x resistant), 25-fold in DC-3F/VCRm (550x resistant), and 75-fold in DC-3F/VCRd-5L itself. Hybridization in situ localized amplified genes to the homogeneously staining region (HSR) or abnormally banding region (ABR) of VCR-resistant lines and permitted identification of short HSRs and/or ABRs on chromosome 1 (at q26) in the AD- and DM-resistant cells and at 2 sites, 1q43 and 2q38, in a sixth line, CLM-7/AD XV (415x resistant). The native pVCR5L-18 gene was localized to chromosome 1q; 13% of the total 660 chromosomal grains from 195 cells were over region 1q24-1q32 with a clear peak at band 1q26. Thus, all 6 cell lines amplified pVCR5L-18 genes albeit at levels not closely proportional to degree of resistance. In contrast to results obtained with dihydrofolate reductase gene amplification in DC-3F cells, amplification of the pVCR5L-18 gene in two of the lines appears to have occurred in loco.

A124 A CELLULAR BASIS FOR NON-SPECIFIC, DECREASING TUMOR RESPONSIVENESS TO THERAPY. John E. Byfield, Daniel Freeman Hospital, Inglewood, Ca. 90301.

Chemotherapy (CT) resistance in individual cancer cells derives from several mechanisms including gene amplification, mutations, etc. operating through population selection pressures & sometimes producing cross-resistance. However, the equally commonly observed cross-resistance seen between modalities, e.g. lower CT responses in previously radiated vs. non-radiated tumors is not easily explained by these mechanisms. The author previously described a model of tumor response based on embryologic organ development and function which dictate post-natal cell renewal mechanisms (Int. J. Rad. Biol. 46:642, '84). The model categorizes tumors into groups with predictable stem cell concentrations. Tissues like skin, mucosa, etc. are hierarchical cell renewal systems while secretory organs & mesenchymal tissues are not. Their derivative tumors then contain comparable differences in clonogenic cells. Tumors from hierarchical cell populations are more "responsive" because any observed "response" is amplified by death of non-clonogenic cells. The model may further explain the non-specific decline in response to therapy seen clinically if one postulates that the stem cells of hierarchical cell tumors variably retain the binary capacity for differentiation basic to true stem cells. The degree to which this function is lost can be termed "slippage". E.g. total slippage in an epidermoid clone would produce an "anaplastic" tumor while little slippage produces a well-differentiated cancer. The effect of any cytotoxic selective pressure is then to enhance the growth of cells with high slippage which in turn increases the concentration of stem cells. This will affect any serial treatment response. The full model will be presented for consideration and comment.

A125 MECHANISM OF ACTION OF CALCIUM CHANNEL BLOCKERS ON MULTI-DRUG RESISTANT CELLS, Danielle F. Cano-Gauci and John R. Riordan, Hospital for Sick Children and University of Toronto, Canada M5G 1X8

Calcium channel blockers (CCB) such as verapamil and nifedipine and some calmodulin inhibitors such as trifluoroperazine have been employed previously in combination with anthracyclines or Vinca alkaloids to improve killing of multidrug resistant (MDR) cells. This effect seemed to correlate with an augmentation of cellular drug accumulation which CCB cause. We now have investigated the influence of CCB on the toxicity and accumulation of daunomycin, vinblastine and colchicine by MDR mouse, CHO and human cells. Several unexpected findings were made. First, CCB augmented the accumulation of some drugs to a much greater extent by sensitive cells than by resistant cells. This occurred despite the fact that verapamil together with daunomycin was much more toxic in MDR cells than sensitive cells. Second, verapamil alone was very much more toxic to MDR cells than to sensitive cells, i.e. MDR cells are collaterally sensitive to verapamil. Third, the accumulation of ^{3}H verapamil by MDR cells is diminished compared to that by sensitive cells to about the same extent as is the accumulation of the cancer drugs. Fourth, the effect of CCB on drug accumulation is mimicked by the non-ionic detergents, Triton X-100 and Lubrol WX. Fifth, CCB were essentially without effect on ^{45}Ca equilibration with either sensitive or resistant cells. These findings suggest that CCB act preferentially on MDR cells, not primarily by enhancing accumulation of other drugs, rather by a specific perturbation of the membrane of MDR cells not related to Ca^{2+} movements. Thus further exploitation of CCB in cancer chemotherapy may derive from developments based on their action as highly specific membrane active agents to which MDR cells are collaterally sensitive. (Supported by the MRC of Canada).

A126 ORGAN SPECIFIC ADHESION OF MOUSE B16 MELANOMA CELLS. R.F. Cerra and R.B. Natale, University of Michigan, Department of Internal Medicine, Ann Arbor, MI 48109

Considerable evidence exists to support the concept that some circulating tumor cells are able to recognize, adhere, invade, and proliferate at distant organs in a specific manner. One mechanism by which metastatic cells arrest in specific organs is by recognition of or an increased affinity for the extracellular matrix. We have developed an in vitro model to examine the molecular basis of specific organ recognition using metastatic variants of the B16 melanoma and organ specific biomatrix. The biomatrix was prepared by extracting murine organs in high salt followed by digestion with DNAase and RNAase. After repeated washes, the residue was frozen, pulverized into a fine powder, suspended in water and coated onto tissue culture plastic. Metabolically labeled B16-F1 (low lung colonizing), B16-F10 (high lung colonizing) and B16-BL6 (high lung colonizing, highly metastatic) were allowed to attach to the biomatrix-coated plates for various times either with continual shear forces or with only a brief shear pulse applied. Following several PBS (with Ca, Mg) washes, the attached cells were solubilized and the amount bound calculated. All three lines adhered better to lung biomatrix than either kidney or liver biomatrix. The B16-F10 and B16-BL6 line, however demonstrated a 2-fold greater adherence to lung biomatrix than the B16-F1 line. A non-metastasizing control line bound equally well to all three of the biomatricies. These derived biomatricies possess characteristics recognized as organ-specfic by metastatic tumor variants and can now be used to biochemically analyze the organ recognition and adhesion mechanism.

A127 ANTIGEN EXPRESSION AND SENSITIVITY TO HYPOTONIC LYSIS IN TUMOR PROGRESSION, Donna A. Chow, Garth W. Brown and Thomas P. Lesiuk, U of Manitoba, Wpg. Man. Canada R3E 0V9

Our previous investigations of the progression of threshold SC tumor inocula in syngeneic mice suggested that the tumors which grew out at the injection site in only 20-30% of the animals, were more heterogeneous and less sensitive to in vivo and in vitro parameters of natural resistance (NR) (Chow, D A et al Int J. Cancer 31:99 1983). Such tumors exhibited higher tumor frequencies and correlating reductions in sensitivity to natural antibody (NAB), NK cells and NR measured as ^{131}IUdR labelled tumor elimination. This selection for resistance was thymus independent and dose dependent supporting the idea that NR mechanisms exerted the selection pressure against the small SC tumor inocula. An examination of the resistant phenotype revealed that the more aggressive in vivo grown cells were less able to inhibit or absorb NK cell activity and bind NAb measured through fluorescence analysis although they could not be distinguished from the starting clones in absorbing NAb for complement mediated lysis. The in vivo grown cells also exhibited a reduced sensitivity to hypotonic lysis which was not detectable after preincubation at 4°C or upon exposure to azide, procedures which reduced the lysis of the starting clones. The differential susceptibility of the in vivo grown cells was increased to control levels by treatment with cycloheximide or colchicine. These studies suggest that reduced antigen expression for binding of NK cells and certain NAb in addition to a decreased sensitivity to lysis associated with a reduced autolytic process and an increased counterlytic mechanism contribute to this resistant phenotype which may characterize tumors that arise in the natural course of neoplastic development. Supported by the NCI of Canada.

A128 Protein-phosphorylation in plasma membrane of normal and neoplastic rat liver. Jon G. Church, Basil D. Roufogalis and Antonio Villalobo. Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

Highly purified plasma membrane fractions from normal rat liver, regenerating rat liver (24 hours after partial hepatectomy) and an ascites form of the transplantable rat hepatocarcinoma, denominated AS-30D, were used in this study. Phosphorylation experiments were performed with [γ^{32}P]ATP and [γ^{32}P]GTP in the presence and in the absence of the protein kinase activators, cyclic-AMP, cyclic-GMP, calcium ion and calmodulin. Vanadate (10 to 500 μM) was used as an inhibitor of phosphoprotein phosphatase(s). Control experiments were performed in the presence of [α^{32}P]ATP to distinguish nucleotide binding from the transfer of phosphate to aminoacid residue(s). The ^{32}P-labelled membrane proteins were separated by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate, and autoradiographs were obtained. We observed general hyperphosphorylation of the tumor plasma membrane proteins and in addition, of chloroform-methanol (2:1 v/v) extractable component(s) of less than 10 K daltons. We have also identified, in the normal plasma membrane, proteins in which ^{32}P-labelling increases (130 K, 123 K, 111 K and 86 K daltons) or decreases (16.5 K daltons) in a Ca^{2+}(+ calmodulin)-dependent manner. In contrast, in the tumor plasma membrane, only the ^{32}P-labelling of a single protein (approximately 23 K daltons) was increased in a Ca^{2+} (+ calmodulin)-dependent manner. (Supported by a research grant from the Cancer Research Society, Inc., Montreal, to A.V.)

A129 GENE AMPLIFICATION AND METASTASIS: C.Cillo, V.Ling and R.Hill; Ontario Cancer Institute; 500 Sherbourne Street, Toronto, Ontario, CANADA M4X 1K9.
Drug resistance and metastasis are common characteristics of advanced cancer. Resistance to certain drugs frequently occurs in tumor cell populations at high rates as a result of gene amplification. Recent studies have demonstrated that metastatic variant cells are generated randomly at high rates within tumor cell populations. In particular it was shown that the highly metastatic cell line B16F10 generated metastatic variants at a higher rate (~5x 10^{-5}/cell/gen) than the less metastatic cell line B16F1 (~1x 10^{-5}/cell/gen)(Science 224:998-1001, 1984). The high rates of generation of metastatic variants suggest the possibility that the variants may originate from gene amplification events. If this is correct we might expect that cell lines, which have different rates of generation of metastatic variants, would also have different rates of generation of drug resistant variants. We tested this prediction by examining the two cell lines B16F1 and B16F10 for resistance to the drugs methotrexate, N-(Phosphonacetyl)-L-Aspartate (PALA), and adriamycin. Resistant variants to these drugs usually develop as a result of gene amplification. Our results indicate that the rate of generation of drug resistant variants is consistently higher in B16F10 than in B16F1 cell populations. In contrast the two cell lines are similar in their resistance to ouabain, a drug to which resistance usually develops by mechanisms not involving gene amplification. Preliminary studies using Southern blot analysis have demonstrated the amplification of the DHFR gene in methotrexate resistant cells. These results suggest strongly that a gene amplification process is involved in the generation of a metastatic phenotype in B16 melanoma cells. -Supported by NCI(C),MRC(C) and OCTRF.

A130 ISOLATION OF cDNA CLONES FOR THE mdr1 (MULTIDRUG-RESISTANCE) GENE, Douglas P. Clark*, Nancy Richert, Tito Fojo, Igor Roninson**, Ira Pastan and Michael M. Gottesman, *Howard Hughes Medical Institute Research Scholar, Laboratory of Molecular Biology, NCI, NIH, Bethesda, MD 20892 and **Center for Genetics, University of Illinois College of Medicine at Chicago, Chicago, IL 60612

The development of resistance to chemotherapeutic drugs is a common problem in cancer treatment. We have developed a series of multidrug-resistant human KB carcinoma cells selected independently for resistance to colchicine, Adriamycin and vinblastine, which show cross-resistance to the selecting drugs as well as actinomycin-D and vincristine. Each of these cell lines carries amplified sequences, some of which have been cloned. One clone recognizes a gene, called the mdr1 gene, which is expressed at high levels in several human multidrug resistant cell lines (see also abstracts by Roninson et al. and Pastan et al.). To isolate cDNA clones for the mdr1 gene, we have constructed a lambda gt11 cDNA library with cDNA from the multidrug resistant cell line KB-C2.5 (selected in 2.5 ug/ml colchicine). The library was screened with a human mdr1 genomic probe. Several large cDNA inserts have been isolated and are being analyzed.

A131 EVIDENCE FOR ALTERED DRUG ACCUMULATION IN MEMBRANE VESICLES FROM MULTIPLY DRUG RESISTANT KB CELLS. M. M. Cornwell, M. M. Gottesman, and I. Pastan, Laboratory of Molecular Biology, NCI, NIH, Bethesda, Maryland 20892.
We have isolated human KB carcinoma cells resistant to high levels of colchicine which are cross-resistant to vinblastine, vincristine, Adriamycin, and actinomycin-D. To determine the mechanism of reduced drug accumulation in these cells we measured ^{3}H-vinblastine (^{3}H-VBL) accumulation in membrane vesicles made from drug-sensitive (KB-3-1), drug-resistant (KB-C4) and revertant (KB-R4) cells (Akiyama et al., Somatic Cell and Molecular Genetics 2, 117, 1985). Membrane vesicles from drug resistant KB-C4 cells accumulated 8-fold more ^{3}H-VBL than vesicles from the parental KB-3-1 or revertant KB-R1 cell lines. No difference in accumulation of ^{3}H-dexamethasone, to which all cells are equally sensitive, was observed. No differences in tubulin content were detected. As shown for whole cells, differences in ^{3}H-VBL accumulation in vesicles were eliminated by the addition of 10 µg/mL verapamil. ^{3}H-VBL accumulation in KB-C4 vesicles was comprised of osmotically sensitive and osmotically insensitive components, whereas ^{3}H-VBL association with KB-3-1 vesicles was osmotically insensitive. Drug accumulation in KB-C4 vesicles was also temperature-dependent and trypsin sensitive. The accumulation of 0.16 µM ^{3}H-VBL by KB-C4 vesicles was inhibited 80% by 1 µM VBL, 85% by 10 µM vincristine, and 30% by 10 µM daunomycin. Colchicine (100 µM), actinomycin-D (100 µM), and dexamethasone (100 µM) did not inhibit ^{3}H-VBL accumulation. These data indicate that membrane vesicles from drug sensitive and resistant cells reflect the differences in accumulation of drugs observed in intact cells.

A132 RADIATION SENSITIVITY OF HETEROGENEOUS MURINE CARCINOMA CELLS IN PROLIFERATIVE vs QUIESCENT STATES, L.A. Dethlefsen, S.E. Sweigert, R.L. Warters and D.N. Ridinger, Department of Radiology, University of Utah, Salt Lake City, Utah 84132.
X-irradiation-induced cytotoxicity of well-oxygenated but nutrient-deprived plateau cells (Q_1) is significantly greater than that for cells in rapid proliferation (P) in two heterogeneous tumor lines; 66 and 67 (D_o's 109 vs 90 and 90 vs 52 rad respectively for 66 and 67 P vs Q_1). Also, the 66 cells are more radioresistant than the 67 cells. In the 67 line, sublethal damage repair studies indicated that in both P and Q_1 cells, recovery was maximum at 3-4 hrs; however, the maximum recovery factor was ≅ 6 and 15 respectively. Potentially lethal damage repair for 67 P vs Q_1 cells had a recovery factor ≅ 2 vs 2.8 respectively at 6 hrs. Also, alkaline filter elution assays showed that: a) Q_1-cell DNA had endogenous strand breaks which did not exist in P-cells, b) radiation-induced damage was ≅ 2x more in Q_1 vs P-cell DNA, and c) the rate of repair of DNA damage in Q_1 cells was ≅ 25% that of P cells. Metabolism studies in 67 cells indicated that both non-protein sulphydryls (NPSH) and glutathione (GSH) levels were ≅ 1.6x higher and [NAD$^+$] ≅ 2x higher in P vs Q_1 cells. The rate of poly(ADP-ribosyl)ation was also ≅ 2.5x faster in P cells. In summary, the molecular mechanisms underlying the decreased cellular radiosensitivity of 66 vs 67 cells is currently not understood. However, the increased cellular radiosensitivity of 67 Q_1 vs P cells may be explained, in part, both by the induction of more DNA damage and its slower repair in Q_1 cells. These phenomena, most likely, are related to the deprived energy state of the Q_1 cells. In contrast, the reduced concentration of endogenous radical scavengers (NPSH, GSH) does not appear to play a dominant role in these aerobic radiation responses.

A133 **A MULTIGENE FAMILY ENCODES P-GLYCOPROTEIN ASSOCIATED WITH MULTIDRUG RESISTANCE**
K. Deuchars and V. Ling. The Ontario Cancer Institute, 500 Sherbourne St, Toronto, Ontario, Canada, M4X-1K9.

Overexpression of a 170,000 dalton membrane glycoprotein, P-glycoprotein, is closely linked to the multidrug resistance phenotype, characterized by cross resistance to unrelated drugs and decreased cellular accumulation of drugs in a variety of mammalian cell lines. Monoclonal antibodies specific for P-glycoprotein have been raised[1]. These were used to isolate a 600 base pair fragment of P-glycoprotein from a λgt11 library of the highly multidrug-resistant Chinese hamster ovary cell line $CH^{R}B30$. Southern blot analyses with this clone, called pCHP1, showed that P-glycoprotein overexpression results from gene amplification[2]. The complexity of the band patterns also suggested that P-glycoprotein is encoded by a gene family. To identify members of this family, eight clones were isolated from a wild-type hamster cDNA library in the Okayama and Berg vector pcD using pHCP1 as a probe. Southern blot analyses with the pcD clones showed that, in addition to a common subset of bands recognized by all the clones, there existed clone-specific bands. These bands probably represent DNA sequences unique to different members of the P-glycoprotein gene family. Sequencing of the 3' ends of these clones has shown that they represent three members of the P-glycoprotein gene family. (Supported by the NCI of Canada and the NIH of the USA.)
1. Elliott, E.M. et al., (1985) Mol. Cell. Biol. 5:236-241
2. Kartner, N. et al., (1985) Nature 316:820-823
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A134 H-RAS TRANSCRIPTIONAL ACTIVATION AND METASTASIS FORMATION, Sean E. Egan, Grant A. McClarty, Jim A. Wright, Arnold H. Greenberg, Manitoba Institute of Cell Biology 100 Olivia St., Wpg., Man., Canada, R3E 0V9.

It has been shown that NIH/3T3 but not C127 cells transfected with either HSV DNA or T24 H-ras are capable of forming experimental and spontaneous metastases in nu/nu mice (J. Cell. Biochem.,Supplement 9C, 1985, Abst.#1180,pp20). In our study, C3H 10T½ mouse cells were transfected with T24 H-ras inserted into pSV2neo, and selected in G418 sulphate. G418-resistant colonies were picked with both refractile and flat morphology. The morphologically transformed colonies gave rise to highly tumorigenic and metastatic lines (in nu/nu and C3H mice), whereas the phenotypically flat NR3 cell line was poorly tumorigenic and produced only rare experimental lung metastases. Four cell lines derived from these NR3 metastases (NR3.1L-A,B,C,D), were highly tumorigenic and metastatic. Four out of five of the original transfectants contained elevated levels of H-ras transcripts by Northern blotting, whereas NR3 had very low levels (comparable to 10T½). The four NR3.1L metastases expressed high levels of H-ras, perhaps indicating that selection for transcriptionally activated cells had occurred during metastases formation. In addition, four radiation induced transformants of 10T½ which were tumorigenic in C3H mice, were not metastatic. No activated ras sequences were detected in these lines. These results indicate that transcription of activated H-ras sequences in 10T½ transfectants can result in tumorigenic transformation as well as expression of the metastatic phenotype. We speculate that expression of activated ras may be necessary but not sufficient for development of the metastatic phenotype. (Supported by the C.H.R.F. and M.H.R.C.)

A135 MOLECULAR BIOLOGY, CYTOGENETICS AND IMMUNOLOGY OF MURINE LEUCEMIC CELLS ARISING IN VITRO FROM CYTOTOXIC T CELL CLONES, Jörg T. Epplen, Hans G. Simon, Ute Hochgeschwender and Markus M. Simon, Max-Planck-Institute for Immunobiology, Freiburg, FRG.

Cloned H-Y-specific cytotoxic T cells (CTLL.1) from female mice change their specificity (CTLL.2,3) in longterm culture and specifically lyse P815 target cells. This switch is correlated with novel gene rearrangements and also with altered expression of the α-, ß-, and γ-chain genes coding for the antigen receptor. Subsequent loss of lectin mediated cytotoxic potential in derived variants (CTLL.4) is accompanied by T-cell growth factor independence reflecting in-vitro transformation. The level of dedifferentiation of CTLL.4 is reminiscent of fetal thymocytes with respect to the expression of antigen receptor genes. Injecting CTLL.4 i.p. into mice generates lymphomatosis with metastases in lymphatic and extra-lymphatic tissues. Tumor cells (CTLL.5) share the characteristics of CTLL.4. The ß-chain genes of CTLL.1,3,5 have been cloned and sequenced from cDNA and genomic libraries revealing the gene rearrangement in vitro and the novel expression of a Vß gene. This Vß gene is highly homologous to another found in human T cell leucemias. The development of cancer cells in-vitro was monitored cytogentically and the typical karyotypic evolution from euploid CTLL.1 to aneuploid CTLL.3,4,5 with chromosome translocations was observed. Parallel studies on the expression of a number of relevant oncogenes revealed no gross changes comparing CTLL.1 to 5. Expression of Moloney murine leucemia virus and mouse mammary tumor virus RNA is not altered during the development of CTLL.1 to CTLL.5. Attempts to generate antibodies against the variable part of the T cell receptor ß chain will be reported as well as functional studies. The results are discussed with respect to persistent stimulation of T cells in-vivo in antigen specific responses and autoimmunity.

A136 THE 5kB mRNA OVEREXPRESSED IN MULTIDRUG RESISTANT CELLS:IN VITRO, IN VIVO OBSERVATIONS AND MOLECULAR CLONING, Gros,P.,Croop,J*,Mukayama,T*,Fallows,D*,Housman,D* Dept of Biochemistry, McGill University, Montreal, Canada;(*)Dept Biology, MIT, Cambridge, Ma,02139. We have observed that multidrug resistance (MDR) is associated in 77A, LZ and C5 hamster cells with amplification of a common genomic domain(1). We have cloned from LZ cells a large portion of this domain and showed that it contains a gene encoding a 5kb mRNA whose level of expression directly correlates the level of resistance of MDR hamster cells(2). Using in vivo and in vitro models, we extend and precise our observations on the association of the 5kb mRNA with MDR. We report that MDR phenotype can be transfered from LZ cells to drug sensitive LTA mouse cells by metaphase chromosome transfer. Southern analysis shows that 4 separate transferants carry 5 to 10 copies of the hamster gene on a mouse background. Northern analysis shows that transfer of resistance is linked to elevated levels of the 5kb mRNA in these clones. We have also studied the MDR phenotype of an in vivo derived mouse leukemic line, L1210DN. This cell line was selected in vivo for Daunorubicin resistance and stably maintains drug resistance (Daunorubicin, Adriamycin, Cochicine) during in vivo passage in the absence of drug. Southern analysis shows that L1210DR carries 30 copies of a mouse homologue to the cloned hamster gene. Northern analysis shows that this amplified gene encodes a 5kb mRNA highly expressed in these cells. These data strongly suggest that the 5kb mRNA plays a central role in MDR, both in vivo and in vitro. We have now isolated two populations of cDNA clones complementary to the 5kb mRNA. Both of these clones are shown to be homologous to hamster DNA of the genomic domain cloned from LZ and homologous to the 5kb mRNA overexpressed in MDR cells.

A137 DNA AMPLIFICATION AND ITS ROLE IN KARYOTYPIC HETEROGENEITY AND TUMOR PROGRESSION
Michael J. Higgins, D.L. Reimer, M.R. Hough, W. Abramow-Newerly, J.C. Roder, I. Shtromas, B.N. White, and J.J.A. Holden. Queen's University, Kingston, Ont., Canada.
We have been studying the role of DNA amplification in tumor progression using the human melanoma cell line MeWo. The earliest available passage of this cell line contains seven cell types, three of which have long homogeneously staing regions (HSRs). They stain with distamycin A/DAPI and silver suggesting that they originated from the centromeric heterochromatin and NOR regions of chromosome 15. These HSR-containing cells were strongly selected for during growth in nude mice. Furthermore a hamster-human hybrid cell line carrying an HSR exibited enhanced tumorigenicity compared to an HSR-lacking hybrid line with a similar human chromosome background. Examination of other MeWo cells identified several new chromosomal rearrangements, the majority of which involved the NOR-containing sequences and/or the distamycin A/DAPI positive region of chromosome 15. We estimate that the rDNA and heterochromatic DNA together account for about 80% of the total in MeWo HSRs. While it is unlikely that these sequences contribute to tumor progression directly, our data suggest that they do impart a specific chromosomal instability to MeWo cells which generates cell heterogeneity. The strong correlation between enhanced tumorigenicity and the presence of HSRs in MeWo and hybrid cell lines has prompted us to search for DNA sequences conferring the tumorigenic phenotype. DNA transfection experiments in conjuction with the nude mouse tumorigenicity assay are in progress. This work was supported by the National Cancer Institute of Canada.

A138 REVERSAL OF ARA-C RESISTANCE BY TREATMENT WITH DIHYDRO-5-AZACYTIDINE(DHAC)
V.Avramis, R.Biener, B.Antonsson, and J.Holcenberg, Childrens Hospital of LA, USC School of Medicine, Los Angeles,CA 90054
DHAC, a hydrolytically stable derivative of 5-azacytidine, causes hypomethylation of DNA and has antileukemic activity. We have previously reported that administration of a LD10 dose of DHAC to mice bearing L1210 ara-C cells can partly restore their sensitivity to ara-C(Proc AACR 25:333,1984). The untreated cells lack deoxycytidine kinase(dck) and do not form ara-CTP after in vitro incubation with 1 mM ara-C. 48 hr after the DHAC treatment these cells express approximately 10% of the ara-CTP formation of the native L1210 cells. We have now treated human CEM cells that are deficient in dck activity. These cells also increase ara-CTP formation and show a large decrease in DNA methylation after in vitro DHAC treatment. Cloned revertants form as much ara-CTP after incubation with ara-C as the native CEM/0 cells. A clinical protocol has been initiated to treat children with acute lymphocytic leukemia resistant to high dose ara-C. These patients receive a 5 day continuous infusion of 5-azacytidine and then are retreated within 5 days with another course of high dose ara-C. The 2 patients studied to date have cleared their blood of lymphoblasts but both died of infection. One patient had no evidence of leukemia at autopsy. His lymphoblasts showed a decrease in DNA methylation after the 5aza-C treatment. Further studies are underway to define the mechanism of this reversal of resistance and define its clinical usefullness.

A139 NIFEDIPINE AND CISPLATIN: SYNERGISTIC ANTITUMOR EFFECTS. K. Honn, J. Taylor, J. Jacobs & J. Onada, Wayne State University, Detroit, MI 48202.
The problems of drug resistant tumors and their usual catastrophic consequences for the patient are commonly recognized. Efforts to overcome this problem have included manipulation of chemotherapeutic drug(s) dosage, scheduling of, method of administration and the use of multiple antitumor agents as opposed to single drug therapy. A recent development has been the use of a non-cytotoxic drug such as the calcium channel blocker (CCB) verapamil to enhance the antitumor effects of cytotoxic chemotherapeutic agents. Recent reports have documented the efficacy of "enhancement Therapy" (ET) using verapamil in combination with adriamycin, vincristine, etc., against cultured tumor cells in vitro and ascitic and leukemic tumor cells in vivo. Solid tumors and their metastases, however, are the most common of fatal concers; and, ET studies directed against solid tumors and their metastases are virtually non-existent. Therefore, we have examined ET therapy using the CCB nifedipine to potentiate the antitumor effects of cisplatin (CDDP) in vivo against solid primary tumors and their metastases. We found that nifedipine greatly enhanced the antitumor effects of cisplatin against solid subcutaneous murine tumors (>50% reduction vs. CDDP alone) and their spontaneous pulmonary metastases (>50% inhibition vs. CDDP alone) and significantly prolonged survival of ET treated mice as opposed to mice treated with a single drug alone. ET therapy was effective against a murine tumor line (3LL) which is inherently resistant to cisplatin alone and against a cisplatin resistant clonal variant of a cisplatin sensitive cell line (B16a). We suggest that ET therapy using CCB and cisplatin should be evaluated against human solid tumors and their metastases.

A140 The concept of supporting pressure: a help to understand Cancer. SYED A.IMAM, Microbiology Div.,Central Drug Res.Inst.,Lucknow,INDIA. The concept of supporting pressure would encompass the concepts of osmotic pressure, viscosity & surface tension and it would represent the total pressure of molecules, built up as a result of organisation of macromolecules to form a tissue in a body, for instance. The fall of pressure of molecules may result in de-differentiation or possible onset of Cancerous state of growth; differentiation represents a healthy level of pressure of molecules in various organs and tissues. The various levels of pressures of molecules in a living tissue would not remain constant and variation in this would show up as biological variation of properties. The viscosity, surface tension and osmotic pressure may be seen as manifestations of the supporting pressure of molecules which hold together in a cell molecular configuration in health and disease. Thus molecular organisation inside a tissue would be backed by this supporting pressure. It is then possible to conceive of collapse, partial or complete, of this pressure due to a disease like cancer, resulting in disrupting correspondingly the supported molecular structure and vice versa. Thus treatment or cure of cancer for example may be spoken of as bringing back the same levels of supporting molecular pressures which obtain in a healthy state. The pressure of molecules exhibiting itself in the form of for example, osmotic pressure reveals now as a new fundamental in the make up of a cell.

A141 CHEMICAL AND BIOLOGICAL CHARACTERIZATION OF SEMI-SYNTHETIC INHIBITORS AGAINST METASTATIC MELANOMA CELL-DERIVED HEPARANASE. Tatsuro Irimura, Motowo Nakajima and Garth L. Nicolson, Department of Tumor Biology, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

The destruction of microvascular wall basement membrane is required for blood-borne metastatic lung colonization of melanoma cells. Heparan sulfate proteoglycan (HSPG) is an important constituent of endothelial basement membrane, and in fact, HS degradative activities B16 melanoma sublines correlate with their lung colonization activities. We have been attempting to produce inhibitors against HS specific endo-β-glucuronidase (heparanase). We have examined a series of chemically modified heparins and found that some of these derivatives such as I. N-acetylated N-desulfated heparin; II. N-resulfated N- and O-desulfated heparin; and III. caboxy-reduced heparin possessed inhibitory activity with minimal anticoagulant activity. Heparin inhibited release of ^{35}S-labeled HS fragments from endothelial matrix, and also induced release of ^{35}S-labeled macromolecules, presumably HSPG. Whereas compounds II and III were inhibitory against heparanase without causing spontaneous release of HSPG, II and III were inhibitory to the growth of mouse lung endothelial cells, but did not affect the growth of B16-BL6 melanoma cells. They slightly enhanced the initial kinetics of adhesion of B16-BL6 melanoma cells to endothelial matrix. When these substances were incubated with B16-BL6 melanoma cells and injected intravenously into C57BL/6 mice, reduction of the number of lung colony after 21 days was observed.

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A142 EPIGENETIC CONTROL OF TUMOR CELL PHENOTYPE, Raymond J. Ivatt, University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030

Tumor cell heterogeneity is a major complication for effective cancer therapy. This heterogeneity can arise as a consequence of long-term, genetic alterations and can also arise as a result of short-term effects. We have been studying the latter, specifically, the short-term heterogeneity of tumor cell adhesiveness during the cell cycle and in response to cellular contacts. Our studies with the murine teratocarcinoma system have identified a carbohydrate-mediated cellular adhesive system. The system is characteristic of the stem cells and is lost on differentiation. The expression of the carbohydrate moiety is conserved during the cell cycle and is modulated by cellular contacts. In contrast, the expression of the carbohydrate receptor shows a periodic variation during the cell cycle and very little alteration in response to cellular contact. As the carbohydrate is the limiting component, the net result is that cells maintain their cohesiveness during periods of continued proliferation but have decreased adhesiveness as the number of cellular contacts increase. This may provide a homeostatic mechanism for regulating the strength of adhesive contacts. Understanding how intrinsic and extrinsic factors regulate the strength of cellular adhesiveness is an important part of understanding the metastatic phenotype.

Supported by Basil O'Connor Starter Research Grant, March of Dimes 5-365, and Grants CD-153 and BC-426 from The American Cancer Society.

A143 MOLECULAR MECHANISMS OF BLEOMYCIN RESISTANCE IN HUMAN TUMOR CELLS, John S. Lazo and Susan G. Amara, Yale University, New Haven, CT 06510
The mechanisms determining natural and acquired resistance by human tumors to antitumor antibiotics are poorly understood. Using brief mutagenesis and constant exposure to bleomycin (BLM), we have isolated 3 stable BLM resistant clones from cultured human head and neck carcinoma cells (A-253). The resistant phenotype was specific for BLM since no clones displayed cross-resistance to vincristine, doxorubicin, cis-diammine-dichloroplatinum, or x-irradiation. No significant BLM metabolism was detected with intact cells. Two clones had reduced BLM cellular association suggesting altered drug uptake or efflux. The mechanism of resistance for the third clone remains unclear. The identification of a BLM resistance gene (ble) on the bacterial transposon Tn5 suggests that genetic factors may control BLM resistance. Using deletional analysis, we have mapped the location of the ble gene to a 580 bp region between neomycin and streptomycin resistance genes on Tn5. The ble gene has been inserted into an SV40 eukaryotic expression vector and the expression of the gene in stably transfected A-253 cells is being evaluated. The availability of stable tumor cell lines with acquired resistance to BLM should allow further clarification of the molecular basis for antitumor antibiotic drug resistance in malignant human cells.

A144 TIME-DEPENDENT CELLULAR COMPOSITION OF AN ARTIFICIAL HETEROGENEOUS XENOGRAFT COLON TUMOR, John T. Leith, Seth Michelson, Lynn Faulkner, Sarah F. Bliven, and Arvin S. Glicksman, Brown University and Rhode Island Hospital, Providence, RI 02902

Two clonal tumor subpopulations, termed A and D, originally isolated from a human colon adenocarcinoma, DLD-1, have been mixed in varying proportions to create artificial heterogeneous neoplasms. The cellular characteristics of pure clone A and clone D tumors in nude mice were compared to those of 5:95, 50:50, and 95:5 percentage admixtures of clone A and D cells respectively in the initial injection bolus. The time-dependent cellular composition of these neoplasms was measured by enzymatically disaggregating tumors and plating the obtained single cells in vitro for assessment of clonogenic potential. Developing colonies were scored as being either "A- or D-type" on morphological grounds with confirmation of colony ancestry by karyotyping. Therefore, in these experiments the cell yield (cells/mg), colony forming efficiency, and cellular identity of clonogenic cells could be assessed for the different groups as a function of tumor age (to about 60 days postinjection). The data indicate that there is a preferred cellular composition in terms of admixture stability in the retrieval of clonogenic A- or D-type cells for the 5:95 clone A:clone D tumors. In contrast, other admixture compositions are unstable, as the relative percentages of the subpopulations change continually over the life history of the solid tumors. These data, together with classical Gompertzian analysis of growth of these heterogeneous neoplasms, indicate some type of "clonal interactions" and have application to the therapy of solid (heterogeneous) cancers. (Research supported by ACS Grant PDT 243).

A145 Identificaton and Characterization of the Genes Encoding Three Novel MHC Class I Antigens on a C3H Tumor. Richard Linsk*, Hans Stauss#, Hans Schreiber#, and Robert Goodenow*, *Department of Genetics, University of California, Berkeley, CA 94720, #La Rabida Institute, University of Chicago, Chicago, IL 60637

Like most UV-induced C3H fibrosarcomas the tumor 1591 is highly immunogenic and is rejected when transplanted into syngeneic mice. Analysis of 1591 with tumor specific and anti-H-2 monoclonal antibodies revealed that this tumor expresses several novel class I MHC antigens not normally found on the parental C3H tissue, in addition to the normal complement of $H\text{-}2K^k$ and $H\text{-}2D^k$. In order to determine the molecular basis for the expression of these novel class I antigens, we have generated a library from 1591 genomic DNA and have identified three genes whose products account for the unique serological reactivities observed on the tumor. These three genes contain several polymorphic restriction fragments which are detected in genomic Southern blots comparing the class I genes of 1591 to those of normal C3H tissue, suggesting that these genes were generated by multiple recombination events among the endogenous class I genes of C3H. DNA sequence analysis of these three genes suggests that this is indeed the case, since the 3' end of one of the genes is identical to that of the endogenous $H\text{-}2K^k$ gene. Since expression of these novel class I antigens is responsible for the regressor phenotype of 1591, these data suggest that class I gene rearrangements might be intimately associated with the complex immunobiology of UV-induced tumors.

A146 TWO-SITE IMMUNOASSAYS FOR THE MEASUREMENT OF LAMININ IN SERUM AND TISSUE-CULTURE SUPERNATANTS, J.D. Lopes and G. Schechter, Ludwig Institute for Cancer Research, São Paulo Branch, R. Prof. Antonio Prudente 109 - 4, 01509, S. Paulo, Brazil.

Basement membranes seem to play a crucial role on the process of invasion by metastatic cancer cells. They are composed of collagen, elastin, proteoglycans and glycoproteins, and act as boundaries between cells and intersticial stroma. These interactions may be altered in neoplasia, since tumor cells can lyse, sinthesize and stimulate the production of matrix components by bystander cells. Laminin is a 10^6 Kd glicoprotein present in basement membranes and its serum levels could be influenced by those modifications, thus correlating with tumor staging. In the present study we developed a two-site immunoassay to measure concentrations of laminin in sera of different animal species, in tissue culture supernatants and in sera from normal and cancer-bearing patients. Laminin was purified from EHS tumors and used for immunization of rabbits, for the purification of antibodies by affinity chromatography and as standard for the immunoassay. Purified antibodies were used to coat polystirenebeads,were conjugated to peroxidase by the two-step glutaraldehyde method and were labeled with 125_I by the Iodo-Gen method. Normal rabbit serum was passed through an Heparin-Sepharose column and the void volume (laminin-free serum) was used for the preparation of laminin standards. Unknown samples were measured directly against a standard curve obtained for both, radio and enzyme immunoassay. Serum levels varied between species, were very high in mice bearing EHS tumors and seemed to correlate with the staging of various human cancers. In conclusions, levels of circulating laminin could be of importance in the prognosis of human tumors.

A147 LOCALIZATION OF ABUNDANT mRNAs TO INDIVIDUAL LEUKOCYTES FROM MIXED CELL POPULATIONS, Wendy M. Mars, Christine M. Stellrecht, and Grady F. Saunders, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

The myeloproliferative response characteristic of chronic myelogenous leukemia (CML) is believed to be the end result of a primary defect in a hematopoietic stem cell capable of multilineage differentiation. In an effort to better understand this disease, we initially isolated and characterized cDNA clones that represent mRNAs preferentially expressed in cells from CML patients. As the RNAs were originally isolated from heterogeneous cell populations, it is important to determine whether all the cells are producing the RNAs of interest or if only a subset of the population is responsible for the majority of the RNA. Therefore we have utilized hybridization in situ of radiolabeled cDNAs to the cellular RNAs from individual hematopoietic cells. Results in normal bone marrows using two different cDNAs indicate that a minor subset of the population produces a large percentage of the RNA and there is a similar hybridization pattern with both probes. In contrast, the hybridization patterns of these two clones differ within the same leukemic populations. Both are primarily expressed by an eosinophilic cell in the normal populations which suggests this cell may be involoved in the development of CML.

A148 EPIDERMAL GROWTH FACTOR (EGF) RECEPTOR IS INCREASED IN MULTIDRUG-RESISTANT HUMAN NEUROBLASTOMA CELLS, Marian B. Meyers, Barbara A. Spengler, W. P. Violet Shen, Mark E. Furth and June L. Biedler, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Increased levels of [^{125}I]EGF binding were measured in three human neuroblastoma lines independently selected for high levels of resistance to vincristine or actinomycin D as compared to parental cells. Further evidence for receptor modulation was found by quantitative analysis of [^{125}I]EGF affinity labeled receptor, immunoprecipitated receptor, and in vitro phosphorylated immunoprecipitated receptor in resistant and control cells. There is a 7-fold increase in EGF binding in SH-SY5Y/VCR (1333-fold resistant to vincristine) and a 40-fold increase in binding in SH-SY5Y/AD (30-fold resistant to actinomycin D) cells compared to SH-SY5Y (B_{max} for EGF = 1.7 fmoles/10^6 cells). A 25-fold increase was measured in MC-IXC/VCR (6538-fold resistant to vincristine) compared to MC-IXC cells (B_{max} for EGF = 0.3 fmoles/10^6 cells). SH-SY5Y/VCR cells contain double minute chromosomes (DMs) unlike drug-sensitive controls. MC-IXC/VCR cells contain a chromosome bearing a homogeneously staining region (HSR) not present in controls. SH-SY5Y/AD cells have neither DMs nor HSRs but do contain 5 copies of chromosome 7, the chromosome to which the EGF receptor gene has been mapped. Cells of the actinomycin D-resistant line require EGF for growth. Southern blot analysis with ^{32}P-labeled EGF receptor cDNA suggests that the mechanism for receptor increase is not gene amplification. Modulation of EGF receptor expression may be an inherited, epigenetically controlled trait of these multidrug-resistant cells.

A149 METASTATIC HETEROGENEITY OF CELLS ISOLATED FROM A SURGICAL SPECIMEN OF A HUMAN RENAL CELL CARCINOMA, Seiji Naito, Andrew C. von Eschenbach, R. Giavazzi and Isaiah Fidler, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Human renal carcinoma cells (HRCC) and site of implantation in nude mice influence the biologic behavior of this cancer. HRCC obtained from a radical nephrectomy, was dissociated enzymatically. Cells were established in culture or injected s.c. and into the kidney of BALB/c nude mice. The resultant kidney tumor produced liver metastasis and ascites. All tumors growing in nude mice (s.c., kidney, liver, ascites) were also established in culture. These 5 different lines were injected s.c., i.p., i.v., intrasplenically and into the renal subcapsule (RSC) of nude mice and the tumorigenic and metastatic behavior of the cells were determined.

All the lines were tumorigenic after s.c. or RSC injection. However, the metastatic behavior of the HRCC cells was influenced by both the nature of the tumor cells and by the route of injection. In general, cells derived from the liver metastasis produced more metastases in nude mice than other lines. The line established in culture from the primary HRCC was the least metastatic. Even with highly metastatic cells, i.v. or s.c. injection did not yield significant metastasis but the injection of metastatic HRCC cells into the kidney resulted in extensive metastasis. These results indicate that nude mice can be used for the selection of populations of HRCC cells with increased growth and metastatic potential and that the kidney is the most natural and advantageous site for implantation of HRCC.

A150 DIRECT EVIDENCE OF STEM CELL HETEROGENEITY IN A HUMAN BREAST CANCER. R.B. Natale, University of Michigan, Ann Arbor, MI 48109.

A metastatic supraclavicular node from a woman with breast cancer was disaggregated into a single cell suspension, filtered to remove cell clumps and cultured in a two-layer agar system. Dishes were examined immediately after culturing to document single cell distribution and Q 2 days thereafter to monitor clonal growth. On day 11, twenty colonies (>30 cells each) were harvested by microsyringe and individually transferred to microwells. 14 colonies (clones) continued to proliferate for at least 3 months and 7 continued for at least 6 months. 3 clones, carried for 3+ years, have been characterized and possess significant biologic differences. Clone UJ-BRE 1.1 grows as a suspension culture and has a doubling time (DT) of 12 days, cloning efficiency (CE) in agarose of .07%, and modal chromosome number (MCN) of 88. UM-BRE 1.2 grows in suspension and has a DT of 50 hours, CE in agarose of .35% and MCN of 72. UJ-BRE 1.3 grows partially attached and in suspension and has a DT of 36 hours, CE in agarose of .08% and MCN of 80. Attachment to extra-cellular matrix (ECM) was measured using cells radiolabelled with [^{3}H]thymidine. All three clones attached more avidly to ECM derived from rat mammary tissue compared to ECM derived from rat lung, kidney or liver tissue or to tissue culture plastic. UJ-BRE 1.2 had a greater affinity for mammary ECM than UJ-BRE 1.1 or 1.3. Three cell lines, derived from stem cells have been established. We believe that the phenotypic and genotypic differences between these 3 cell lines provide direct evidence of stem cell heterogeneity. (Supported in part by ACS grant CH-231)

A151 EXPRESSION OF AVIAN SARCOMA VIRUS (ASV) GENES IN CULTURED CELLS DERIVED FROM DIFFERENT PERIODS OF TUMOR GROWTH, Louise Poulin and Mark A. Wainberg, Lady Davis Institute, Jewish General Hospital, Montreal, Quebec H3T 1E2

Most tumors induced in chickens by ASV grow actively for several weeks and then regress. We compared tumor cells derived from the «regressor» phase of ASV-induced tumors to those from progressively-growing neoplastic tissue in terms of ASV gene expression. The results indicate that the polyadenylated RNA's extracted from «regressor» tumor cells are much more productive than those from «progressor» sarcoma cells in a reticulocyte lysate in vitro translation system, in terms of both time course and concentration. Those polypeptides which are generated by in vitro translation of «regressor» RNA correspond on SDS-polyacrylamide gels to the known ASV protein species. In contrast, the polyadenylated RNA's obtained from «progressor» sarcoma cells translate much smaller amounts of these same viral proteins. The identity of two of these viral proteins was confirmed by immunoprecipitation of the translation products, using a rabbit serum directed against pp60src and Pr76gag precursor polypeptides. These immunoprecipitations clearly demonstrated that much more pp60src and Pr76gag proteins were produced by the mRNAs of «regressor» cells than of «progressors». Hybridization of viral c-DNA and Northern blots revealed that viral RNA including genomic and subgenomic species (8.6, 5.0 and 3.2 Kb) were present in greater amounts in the cytoplasm of «regressor» as compared to «progressor» tumor cells. The results support the notion that extra copies of viral RNA including subgenomic species may accumulate in the cytoplasms of «regressor» tumor cells, with a subsequent increase in levels of translated viral proteins.

A152 ENHANCED EXPRESSION OF c-myc ONCOGENE IN MAMMARY ADENOCARCINOMAS. Martine Guérin and Guy Riou. Laboratoire de Pharmacologie Clinique et Moléculaire, Institut Gustave Roussy, 94805 Villejuif cedex (France).

The c-myc oncogene was characterized and its expression analysed in 32 mammary adenocarcinomas and in 2 benign breast tumors from 34 untreated patients. The tumor specimens were obtained by surgical excision. Southern blot hybridization experiments have demonstrated amplification of the oncogene (3, 5 and 30 fold) in only 3 carcinomas. The analysis of DNA preparations by several restriction enzymes does not permit to conclude to a c-myc gene rearrangement.

The analysis of total RNA by Northern blot revealed the presence of a 2.4 kb c-myc RNA band. In 7 out of 10 carcinomas from patients with lymph node metastases the level of c-myc expression evaluated by dot analysis was 4 to 14 fold greater than in normal human tissues (lung, thyroid, lymphocytes, uterine cervix). In only 5 out of 22 carcinomas from patients without lymph node metastases the level of c-myc expression was also elevated (4 to 10 fold). The level of c-myc expression was not significantly enhanced in the 2 benign breast tumors. These results suggest that the c-myc oncogene is activated in mammary cancers of bad prognosis.

A153 GENETIC REARRANGEMENT AND THE GENERATION OF METASTATIC VARIANTS IN B16 MURINE MELANOMA AND HUMAN ASTROCYTOMAS IN CULTURE, G.V. Sherbet, M.S. Lakshmi and G. Hunt, Cancer Research Unit, University of Newcastle upon Tyne, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, England.

The generation of the metastatic phenotype has been attributed to genetic instability and mutation rate is greater in tumours with high metastatic potential compared with corresponding tumours with low metastatic potential [1]. Translocation of chromosomal segments is closely involved with the activation of certain oncogenes in leukemias and lymphomas [2]. We have therefore investigated the relationship between genetic instability indicated by the occurrence of spontaneous sister chromatid exchanges (SCE) and metastasizing ability of B16 melanoma high metastasis variant BL6, low metastasis variant F1, and two cell lines of human astrocytomas, tumours not known to show spontaneous extracranial metastases. Approx. 30% of BL6 cells, 3% of F1 and 2 - 4% of astrocytoma cell populations showed SCE. The cell lines also differed, but not significantly, in the number of SCEs/chromosome. Apparent mutation rates calculated using the Poisson method assuming that mutational events occurred as the result of the chromosomal rearrangements were: F1, 1.18×10^{-5}; BL6, 5.82×10^{-5}; astrocytoma lines IJK_t and G-UVW, 1.33 and 0.89×10^{-5}/cell/generation respectively. These experiments show that genetic rearrangement may cause the generation of variants with metastasizing ability.

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Supported by the North of England Cancer Research Campaign.

A154 The anti-prostatic tumor drug estramustine binds microtubule-associated proteins(MAPs). M.E. Stearns and K.D. Tew. Fox Chase Cancer Center, Phila., Pa..

Estramustine, 17 B-estradiol 3N bis(2-chloroethyl) carbamate, is an antimitotic agent used to treat advanced prostatic cancer. We have investigated the effects of EM on microtubule proteins of brain tissue, human prostatic tumor DU 145 cells and fish chromatophores. ^{3}H-EM binds several high molecular weight microtubule associated proteins (HMW-MAPs) which co-isolate with taxol stabilized microtubules. Immunocytochemical studies with anti-tubulin antibody indicate 120 uM EM will disrupt microtubules in vivo and in detergent cytoskeletons of DU-145 cells and fish chromatophores. Fluorescent microscopic studies show that a fluorescently tagged analogue of EM, dansylated-EM or DNS-EM, will bind taxol stabilized microtubules of erythrophore and sperm axonemes cytoskeletons. If HMW-MAPs and dynein are stripped from the microtubule structures with detergent, salt extraction or trypsinization, the binding affinity of DNS-EM is dramatically reduced. The most striking effect of EM is to inhibit motility in taxol stabilized cell models which are permeabilized with digitonin. At 60-120 uM levels, EM rapidly inhibits transport of pigment granules in chromatophores and axonemal beating by sperm tails. The effects are rapidly reversed with washing in fresh ATP-solutions. The data indicates that HMW-MAPs function to stabilize microtubules and promote organelle transport in vivo. We propose that estramustine represents a novel class of anti-mitotic drugs which have a high affinity for HMW-MAPs and dynein. Helsingborg Research Foundation Grant.

A155 DRUG RESISTANCE IN HUMAN LUNG CANCER CELL LINES. P.R. Twentyman, G.A. Walls, J.G. Reeve and G.L.E. Koch*. MRC Clinical Oncology and Radiotherapeutics Unit and *Laboratory of Molecular Biology, Hills Road, Cambridge, England.

We have taken a number of approaches to obtain radiation or drug resistant variants of human lung cancer cell lines as a basis for mechanistic studies of therapeutic resistance. Multiple high dose treatment of small cell lines with either X-rays, CCNU, vincristine (VCR) or adriamycin (ADM) did not produce major changes in the sensitivity of the regrowing population to the treatment agent. Multi-drug resistant variants of three cell lines (one small cell, one adenocarcinoma, one large cell) have, however, been isolated by growth in increasing concentrations of ADM. Each of the lines is also resistant to VCR and to colchicine and shows reduced ADM content following 1h exposure compared with its parent line. Resistance could be partly overcome by the use of the calcium transport blocker, verapamil (VRP). In the resistant small cell line, the resistance factor is much lower for aclacinomycin A than for ADM and this resistance can be totally removed by VRP.

A number of changes in cellular protein composition have been observed in the resistant lines (and also in three multi-drug resistant rodent cell lines). The significance of these changes will be discussed.

A156 FIVE GENES ARE AMPLIFIED IN THE MULTIDRUG RESISTANT CHINESE HAMSTER OVARY CELL-LINE CHrC5. A.M. van der Bliek, M.H.L. de Bruyn, T. van der Velde-Koerts and P. Borst, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

Multidrug resistance may limit the effectiveness of cancer chemotherapy, because the surviving cells can become cross-resistant to drugs differing both in target and chemical structure. The complexity of the resistant phenotype, apparent in the different patterns of cross-resistance, suggests that several components are involved. The CHO cell-line CHrC5 (Riordan et al., Nature 316, 817) selected with colchicine was used to construct a cDNA library. Clones representing overexpressed mRNAs were isolated by differential screening with cDNA probes from the parental (AUX B1) and the resistant (CHrC5) cell-line. Based on RNA blot hybridizations, showing prominent transcripts ranging from 700 to 4500 nt, we conclude that our clones are derived from five overexpressed genes. One clone, which cross-hybridizes with the recently reported cDNA encoding part of a 170 kD P-glycoprotein (op.cit.), sees Eco RI fragments which are differentially amplified in CHrC5 DNA (10 and 30 X). The other four sets of clones recognize distinct genes which are amplified 10 or 30 x. The amplified DNA was analyzed by pulsed field gradient gel electrophoresis after digestion with restriction enzymes that cut infrequently in the mammalian genome. Two genes are coamplified on two Sfi I fragments of 190 and 300 kb. The other three genes, of which one is the P-glycoprotein, reside at least in part on a common 440 kb Sac II fragment. Our observations are compatible with linkage of the genes in one large (> 600 kb) domain of which a segment has been triplicated prior to amplification of the whole domain.

A157 DECREASED EXPRESSION OF pp60src AND ASSOCIATED KINASE ACTIVITY IN REGRESSING NEOPLASMA INDUCED BY AVIAN SARCOMA VIRUS (ASV), Mark A. Wainberg and Louise Poulin, Lady Davis Institute, Jewish General Hospital, Montreal, Quebec H3T 1E2

Tumor cells which are derived from regressing avian sarcomas contain approximately one-fourth as much pp60src kinase activity as that found in progressively-growing tumor cells, as determined by radioautography in a standard IgG heavy chain ^{32}P transfer assay. Puromycin treatment of cultured sarcoma cells revealed that the half-lives of kinase activity in progressively-growing and regressing cells were 3.2 h and 8.5 h, respectively. Pulse-chase studies, in which ^{35}S-methionine-labelled pp60src was directly precipitated by rabbit anti-pp60 immunoglobulin, indicated that the half-lives of pp60 itself in these two cell types were 1.5 h and 6 h, respectively. We also asked whether differences might exist in an in vitro protein synthesis system. Significant differences between the two cell types were not obtained using this approach. Finally, the addition of cytosolic extracts from regressing tumor cells to those of progressively-growing tumor cells caused a diminution in the levels of detectable pp60src and associated kinase activity in the latter tissue. These findings suggest that a correlation exists between levels of expression of pp60src and the ability of avian sarcomas to continue to enlarge, and that sarcoma cells which derive from the regression phase may contain enzymes or other factors which degrade and/or inhibit pp60src and its kinase activity. Supported by the Medical Research Council of Canada.

A158 DNA-MEDIATED TRANSFER OF HEAT-RESISTANCE PHENOTYPE, Tien-wen Tao, Charles Hsu and Shao-ling Leu, Stanford University, Stanford, CA 94305

An extensive library of stable heat-resistant (HR) variants was isolated from mouse melanoma cells by repeated heating cycles at 43°C and at 45°C in culture. Stepwise heating induced a progressive increase in resistance to heat. The resistance phenotype was expressed not only in vitro as cultured cells but also in vivo as tumor nodules. While the heat-shock-proteins did not seem to be responsible for the HR expression, the HR phenotype however appeared to affect the regulation of the heat-shock response: non-constitutive hsp68 was preferentially induced in the heat-sensitive (HS) cells while constitutive hsp70 was more dominant in the HR cells. On the other hand, HR variants showed a dramatic alteration in the adhesive properties, which suggest that the cell surface may play an important role in the HR behaviour. To understand the molecular and genetic basis for the HR phenotype, total cellular DNA from HR cells was transfected into HS cells. A direct heating strategy was used to select for transformants, which expressed the HR phenotype: heating at 45°C for 60 min resulted in 1 surviving cell out of a million *untransfected* HS cells, while 100 cells out of a million *transfected* HS cells survived such a heat treatment Experiments are under way to clone for the HR gene and to identify the gene product(s).

A159 MANIFESTATIONS OF DRUG RESISTANCE IN BREAST ADJUVANT CHEMOTHERAPY TRIALS, William H. Wolberg, University of Wisconsin-Madison, Madison, WI 53792

The careful analysis of adjunctive chemothrapy trial results following breast cancer surgery provide interesting insights into the expression of drug resistance. Patients treated with adjunctive chemotherapy die sooner following recurrence than do non-adjunctively treated patients so adjunctive chemotherapy does not benefit survival even though it delays recurrence. In most trials, adjunctive treatment delays recurrence by the same amount of time as survival is shortened after recurrence. Patients given the alkylating drug melphalan in adjunctive trials, and particularly in the SWOG trial 7436, have an inordinately poor survival following recurrence which may be due to the development of pleiotropic drug resistance. Such adjunctive trials are the ultimate proving-ground for theories concerning chemotherapy. New concepts must be developed to deal with the problem of drug resistance.

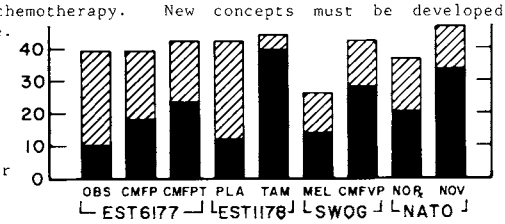

Figure shows months elapsing from diagnosis for the 20th percentile of patients to develop recurrence ■ and the 20th percentile to die (top of bars) in several large studies. The time interval between recurrence and death ▨ is influenced by prior adjunctive therapy and by therapy given after recurrence.

A160 LYMPHOCYTE-ENDOTHELIAL CELL INTERACTION: A MODEL FOR LEUKEMIA AND LYMPHOMA METASTASIS, Nora W. Wu, Robert Bargatze, Larry Weiss, Carlos Garcia, Sirpa Jalkanen, Steve Smith and Eugene C. Butcher, Stanford University, Stanford, CA 94305

Normal lymphocytes recirculate continuously between the blood and lymphoid organs, utilizing specific receptors on the lymphocyte surface which preferentially adhere to post capillary high endothelial venules (HEV) in certain lymphoid organs (eg. peripheral lymph nodes, mucosal associated appendix). The expression of these "homing receptors" is regulated during lymphocyte development and differentiation, controlling the migration and distribution of normal lymphocyte subsets. Using a) Hermes-1, a monoclonal antibody that defines a human lymphocyte surface glycoprotein involved in lymphocyte recognition and binding to HEV and b) an in vitro functional assay of lymphocyte binding to HEV on frozen sections, we have examined a series of malignant and transformed B and T cells, derived from in vivo surgical specimens, frozen patient sample cells and in vitro tumor cell lines. We have found that many neoplastic and transformed lymphocytes express "homing receptors" and interact with HEV in a variety of distinct patterns, often similar to their normal derived counterparts. Children with CALLA+ ALL, who at diagnosis have enlarged peripheral lymph nodes and hepatosplenomegaly,express the antigen for Hermes-1 on their malignant lymphocytes (N=6). Many B cell lymphoma/leukemia patients present with a large solitary mass, usually in the abdomen,without distant metastasis and fail to stain with Hermes-1 (N=8) and 0/4 have the functional ability to bind to HEV of either mucosal or peripheral lymph node specificity. Cells from patients with lymphocytic lymphoma (13/14) stain with Hermes-1 and 3/4 bind well to HEV. Our preliminary data suggests an interesting correlation of the HEV binding abilities of neoplastic lymphocytes with their in vivo growth patterns. The assay of "homing receptor" expression with Hermes-1 and/or with the in vitro functional assay may be useful in the diagnosis and classification of human leukemias and lymphomas, and provides a model which may be useful in predicting their in vivo ability to metastasize hematogenously to various specific lymphoid organs.

A161 GENE DELETION AS A MECHANISM OF ESCAPE FROM IMMUNE ATTACK, Berton Zbar, Yoshiro Tanio, and James Talmadge, NCI-FCRF, Frederick, MD, 21701

We studied models characterized by in vivo selection of tumor populations lacking cell surface transplantation antigens. These tumor populations had deleted genes that encode the relevant cell surface transplantation antigens. In the first model, a cloned line of guinea pig fibrosarcoma cells was infected with an amphotropic murine leukemia virus (MuLV). The retrovirus-infected cell line was heterogeneous in number of proviruses integrated per cell and in retroviral gene expression. When the retrovirus-infected cell line was injected into syngeneic guinea pigs, tumors grew and regressed. Recurrent tumors lacked the MuLV provirus, viral cell surface antigens and specific resistance to retroviral infection. Clones from the retrovirus-infected cell line were evaluated as precursors for tumor recurrence. Under conditions of immune selection a clone that contained a single abbreviated copy of the provirus formed variants that lacked the proviral gene. These variants grew progressively in both nonimmune and virus-immune animals.

In the second model, the growth of B16-BL6 melanoma cells ($H\text{-}2^b$) transfected with a gene encoding $H\text{-}2D^d$ was evaluated in syngeneic nonimmune mice and mice immunized to $H\text{-}2D^d$ antigens. Pulmonary metastases in both nonimmune and immune mice lacked part or all of the transfected plasmid and did not express the $H\text{-}2D^d$ cell surface antigen.

A162 EXPERIMENTAL METASTASIS AS A MODEL OF TUMOR HETEROGENEITY IN HUMAN TUMOR XENOGRAFTS IN ATHYMIC MICE, Robert J. Zimmerman and Elias T. Gaillard; Charles River Biotechnical Services, Wilmington, MA 01887.

The metastatic potential of 12 human tumor xenografts was assayed following tail vein inoculation into 3 to 4 week old gnotobiotic athymic mice in order to study the heterogeneity of these tumors with respect to their lung colonizing ability. Evidence for apparently pre-existing, stable subpopulations capable of rapid lung tumor formation was found in 3/4 melanomas and 2/7 adenocarcinomas of the colon that were tested. In 2 of the melanomas, 5 sublines with the potential to cause host mortality within 4 to 10 weeks post-inoculation of 10^5 cells were identified by repeated cycles of lung colony recovery and re-inoculation. Variants subpopulations from the colon tumors, on the other hand, caused mortality by only about 12 weeks. A renal cell carcinoma, 1/4 melanomas, and 3/7 colon tumors produced lung colonies, but these cells could not be stably isolated. Two of the colon carcinomas did not form lung colonies in the 24 week period of study. These results demonstrated 1) the potential of this model to study aspects of the metastatic process and to identify variant subpopulations in human tumors, 2) that human tumors are heterogeneous in their lung colonizing ability, 3) that both apparently random and non-random processes were involved in the lung colonizing ability of these xenografts, and 4) that dynamic processes may also play a role in tumor heterogeneity.

Late Additions

A163 MOLECULAR STUDIES OF T(6;9) IN ACUTE LEUKEMIA: ROLE OF C-ABL. C.A. Westbrook, M.M. LeBeau, M.O. Diaz, J.D. Rowley. University of Chicago, Chicago, IL 60637.

Human leukemias are frequently associated with specific chromosomal translocations. We have studied in detail one such translocation, t(6;9)(p23;q34) in which there is a reciprocal exchange between chromosomes 6 and 9. The breakpoint on 9, q34, is the same as that in the t(9;22) in CML, suggesting a role for c-abl in this disease. Two patients were studied by in-situ hybridization with a v-abl probe, and showed that the c-abl gene is not translocated from chromosome 9 to 6. As the orientation of c-abl has previously been determined, this indicates that the breakpoint lies 3' of the gene, but Southern hybridization revealed no rearrangements up to 18 kb 3'. In addition to the t(6;9), one patient had a translocation involving chromosomes 4 and 10, t(4;10), present in all cells with the t(6;9). In these cells, a third copy of c-abl was present at the 4;10 junction, on the 4q+ chromosome. Similiar results were found by in-situ hybridization using probes up to 100 kb 5' of c-abl. Thus, the insertion on the 4q+ chromosome is at least 100 kb in length and represents a duplication and translocation of the c-abl region. We next investigated the c-abl protein by immunoprecipitation, and found that it is normal in size and abundance, and does not contain tyrosine kinase activity. In conclusion, we show that the role of c-abl in the t(6;9) is not the same as it is in the t(9;22) of CML, in that the breakpoint is on the 3' end of the gene, and the protein appears unchanged. However, the clinical similarity of these two diseases, and the "double dose" of c-abl in one patient, suggests that c-abl or nearby genes, may be involved. Efforts are now underway to analyze these cells with pulsed field electrophoresis, and to reach the 6;9 junction by "walking" with cosmid libraries.