BIOCHEMICAL AND MOLECULAR EPIDEMIOLOGY OF CANCER

Curtis C. Harris, Organizer

April 6 — 13, 1985

Keynote Address

1306 CANCER: A WORLD-WIDE PERSPECTIVE. Richard Doll, Imperial Cancer Research Fund, University of Oxford, England.

The incidence of cancers of different sites varies from one part of the world to another. Every cancer that is common somewhere is uncommon or rare somewhere else. This variation is mainly due to nurture: that is, to differences in behaviour, exposure to infection or external carcinogenic agents, or to external factors that modify the formation of carcinogens in vivo. There is increasing evidence that most human cancers are the end result of a process that can be interrupted at more than one stage (e.g., by preventing either viral infection or exposure to a chemical agent) and the prevention of a large proportion of currently fatal cancers is now practicable nearly everywhere, by different means in different countries.

The important part played by nurture is compatible with major differences in individual susceptibility to each type of cancer of the order of 100-fold. The discovery of the nature of these differences, would, in some cases, permit more focussed programmes for prevention.

Principles of Cancer Epidemiology

1136 GENETIC ONCODEMES AND ANTI-ONCOGENES, Alfred G. Knudson, Jr., Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

The burden of cancer falls unequally upon a population. Some persons acquire cancer by chance alone, without any particular hereditary or environmental predisposition. The somatic mutation hypothesis implies that background mutation rates would cause an irreducible burden of cancer in a population. A second group has a higher incidence of some cancer(s) because of exposure to environmental agents that increase the probability of occurrence of one or more of the events on the path to cancer. A third group has a higher incidence as a result of genetic predisposition to an increased spontaneous or induced rate of one or more events; xeroderma pigmentosum exemplifies this group. Finally, a fourth group is strongly predisposed to cancer because of inheritance of a mutation that constitutes one of the oncogenic steps. The classes of genes in which these mutations occur are cancer genes and may be relevant to the cancers of all four groups. Each of these four groups may be called an "oncodeme," a demographic unit with a peculiar susceptibility to a particular cancer (1).

The last group is of particular interest because its cancers would seem to identify one or more classes of "cancer genes" that are important in the non-hereditary forms of the same cancers. Evidence has been assembled from the study of retinoblastoma (RB), Wilms' tumor (WT), and neuroblastoma (NB) that two events are necessary in their causation. The first event is germinal in hereditary cases and somatic in non-hereditary cases, whereas the second event is somatic in both. The events were regarded as genetic (including mutation, deletion, and chromosome loss). The second event was regarded as arising de novo or by somatic recombination, and leading to hemizygosity or homozygosity (2). Because the normal alleles of such genes would in effect be anti-oncogenic, such loci were called anti-oncogenes. It was proposed that RB and WT would be the best models for further study, because constitutional deletion cases had provided chromosomal localizations of the relevant genes. Recent research of others has demonstrated that these mechanisms do operate and that such a group of cancer genes, different from oncogenes, exists. This research will be discussed at this meeting.

Important questions remain. Patients with RB seem to belong to either the first or the fourth oncodeme, but might some be in the second or third? Does RB always involve a primary mutation in an anti-oncogene? Do Burkitt's lymphoma patients always belong in the first or second oncodeme, and do their tumors always primarily involve an oncogene? Do the common adult cancers occur in all oncodemes and involve either oncogenes or anti-oncogenes?

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1137 GEOGRAPHICAL EPIDEMIOLOGY AND MIGRANT STUDIES, Calum S. Muir, Chief, Unit of Descriptive Epidemiology, International Agency for Research on Cancer, 150, Cours Albert Thomas, F-69372 Lyon Cédex 08, France.

The cancer scenery of the world is as diverse as other aspects of global topography. Although large areas have neither incidence nor mortality statistics, it has been possible to estimate the world cancer burden as being around 6 million new cases annually. Sites most frequently involved in males are lung, stomach, large bowel, mouth/pharynx and prostate; in females breast, cervix, stomach, large bowel and lung. The lung cancer burden in both sexes is likely to rise till at least 2000, the importance of gastric cancer continuing to diminish (although the commonest cancer today when both sexes are considered). In females breast cancer is likely to rise substantially with cervix cancer decreasing. Examination of regional patterns reveals major differences. In addition to what might be termed region-specific neoplasms such as nasopharynx in Cantonese, oropharynx in the Indian sub-continent, islands of very high oesophageal cancer risk in Iran, North China and parts of France and primary liver cancer in sub-Saharan Africa, South-East Asia and Coastal China, there are major differences in the level of gastric, lung, cervix, breast, large bowel and prostate cancer. To mention but one, the ratio of incidence for prostate cancers runs from 19 in US Blacks, 9 in US Whites, 4 in UK and unity in Japan. The incidence of breast, large bowel and prostate cancer is rising in those populations where risk is currently low.

Such major risk differentials have been interpreted as reflecting differences in the environment using that world in its broad sense of all that impinges on the human organism. That this interpretation is likely to be correct gains support from migrant studies where populations of a given genetic composition change environments. Such studies, exemplified by the classical enquiry of Haenszel and Kurihara, clearly reveal the strong influence of the environment and may offer insight into the possible nature of the agents responsible. Regrettably there has been little attempt to exploit other migrant populations e.g. Indo/Pakistanis in UK, Southern Europeans in Australia, Hispanics in the US. The reasons are many including unwillingness of governments to recognise the existence of migrant populations and of the latter to be counted. Major changes in risk occur for the cancers believed to be linked to lifestyle and diet: their study is likely to be expensive and may have to be of long duration. Similar remarks apply to populations in transition e.g. in Japan.

In summary, geographical differences in cancer occurrence (and change of risk in migrants) provide strong evidence for the environmental origin of malignant disease. Any theory of causation has to be in consonance with these patterns. It is greatly to be regretted that migrants are not more extensively studied.

Genetic Basis of Cancer

1138 EVIDENCE FOR RECESSIVE HUMAN CANCER GENES, William F. Benedict, Department of Pediatrics, Childrens Hospital of Los Angeles, Los Angeles, CA 90027

We and others (1,2) have presented evidence that the gene responsible for retinoblastoma, an ocular cancer of childhood, is recessive and that loss or inactivation of both alleles at chromosomal region 13q14 is a primary mechanism in the development of this tumor (1,3). Such a mechanism is in direct contrast to that proposed for putative human oncogenes. We have also suggested that the retinoblastoma gene is a model for a class of recessive cancer genes that have a "suppressor" or "regulatory" function (3). This class of gene includes the Wilms' tumor susceptibility gene located on chromosome 11p. Recently evidence suggesting the recessive nature of the Wilms' gene has been reported (4).

The oncogene, N-myc, has also been found to be expressed at high levels in retinoblastomas, even when the gene is not amplified (5). In collaborative studies, we are looking at this increased expression at the single cell level both in retinoblastomas containing primarily differentiated or non-differentiated cells and in normal fetal retinal cells.

In this presentation further evidence for the recessive nature of certain cancer susceptibility genes will be presented. Additionally, evidence for a similar mechanism is developing second primary cancers in these same patients will be provided. Further information on the role of the N-myc gene in retinoblastoma and secondary malignancies will also be discussed.

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1139 CYTOGENTICS OF CANCER. Peter C. Nowell, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia PA 19104; and Carlo M. Croce, The Wistar Institute of Anatomy and Biology, Philadelphia PA 19104.

Chromosomal studies earlier provided evidence for the clonal nature of most neoplasms, and for the role of sequential genetic change in tumor progression. Now, in combination with molecular techniques, they are indicating how the function of specific genes (oncogenes) can be significantly altered by chromosomal translocations or by gene amplification, contributing to carcinogenesis. Recent examples from human lymphomas, leukemias, and solid tumors will be cited.

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Growth Factors and Mechanisms of Tumor Promotion and Progression

1140 GROWTH FACTORS AND ONCOGENES, Stuart A. Aaronson, National Cancer Institute, Bethesda, MD 20205

The simian sarcoma virus (SSV) onc gene, v-sis, has been sequenced and its 28,000 dalton product, $p28^{sis}$, identified by means of antisera prepared against small peptides derived from sequence analysis. Computer comparisons of the amino acid sequence of human platelet-derived growth factor (PDGF) with the predicted amino acid sequence of $p28^{sis}$ revealed an extraordinary degree of homology. We have shown that $p28^{sis}$ is processed in vivo in a manner closely approximating that of PDGF and possesses striking functional similarities as well. Finally, nucleotide sequence analysis of the human c-sis proto-oncogene has confirmed that it is the structural gene encoding PDGF-2. We wished to determine whether the normal human c-sis/PDGF-2 coding sequence possessed transforming activity. A human clone, c-sis clone 8, which contains all of the v-sis-related sequences present in human DNA, was shown to be transcriptionally inactive when transfected into NIH/3T3 cells. When placed under the control of a retrovirus LTR, the clone was transcribed but lacked transforming activity. A putative upstream exon was identified by its ability to detect the 4.2 kb sis-related transcript in certain human cells. This exon contained potential translation initiation signals which were not present in the first v-sis related exon of human c-sis. When this putative exon was inserted in the proper orientation between the LTR and c-sis clone 8, the chimeric molecule acquired high titered transforming activity, comparable to that of SSV DNA. These findings establish that the normal coding sequence for this human growth factor has transforming activity when provided necessary signals for transcription and initiation of translation. Thus, our studies provide an experimental basis for the hypothesis that derepression of genes for normal growth factors in cells responsive to their growth promoting activities may play a role in processes leading normal cells along the pathway to malignancy.

1141 GENES AND SIGNAL TRANSDUCTION INVOLVED IN PROMOTION OF NEOPLASTIC TRANSFORMATION, Nancy H. Colburn[1], Michael I. Lerman[2], Thomas D. Gindhart[2], Yoshiyuki Nakamura[1] and Bonita M. Smith[1], Laboratories of Viral Carcinogenesis[1] and Experimental Pathology[2], National Cancer Insititute, Frederick, Maryland 21701

That one or more stages of tumor promotion are irreversible suggests that tumor promoters can act on preneoplastic cells to produce stable switches in gene expression that lead to malignancy. We are testing the hypothesis that sensitivity to promotion of neoplastic transformation in JB6 preneoplastic mouse epidermal cells is determined by genes that are inducible by phorbol esters and other tumor promoters. Recently we have cloned from a library of promotion sensitive (P+) JB6 cells two different genes specifying sensitivity to promotion of anchorage independent transformation (1). Both genes (called pro-1 and pro-2) are independently and equally active when assayed by DNA transfection into promotion resistant (P-) cells. The sequence of pro-1 has been determined and suggests that it may encode a protein of about 7000 daltons. Transient transcription of pro-1 is induced by TPA in JB6 P+ cells.

The biochemical signals that may mediate the promotion process or specifically the regulation of pro gene expression by tumor promoters are currently being investigated. Promotion of JB6 cell transformation apparently requires elevated superoxide anion, as indicated by its sensitivity to superoxide dismutase (2). This superoxide anion requirement occurs only during the first two hours following exposure of JB6 P+ cells to TPA. The JB6 cell transformation process also requires extracellular calcium as indicated by its sensitivity to EGTA. There appear to be at least two extracellular calcium requiring events, one occurring early (minutes) and the other late (days) after TPA exposure. The role of protein kinase C-catalyzed protein phosphorylation in the process is also being investigated (3).

Consideration of these and other findings on genes and signals involved in JB6 cell transformation prompts us to propose a model for pro gene regulation and function(s) in which pro gene products may act as transcriptional regulators or growth factors.

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1142 PHOSPHORYLATION EVENTS IN ROUS SARCOMA VIRUS TRANSFORMED CELLS
R.L. Erikson, J. Blenis, H.P. Biemann and Y. Sugimoto, Department of Cellular and Developmental Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138

It is now generally accepted that the Rous sarcoma virus (RSV)-transforming gene product $pp60^{v-src}$ has protein kinase activity with specificity towards tyrosine residues. In addition we have recently shown that purified enzyme also phosphorylates low molecular mass alcohols and phosphatidylinositol. Since phosphatidylinositol turnover may play an important role in generation of the putative second messengers, diacylglycerol and inositoltrisphosphate, we are also investigating their metabolism in transformed cells and partly purified plasma membranes.

In serum-starved RSV-transformed cells the ribosomal protein S6 remains phosphorylated under the influence of $pp60^{v-src}$ substrate. Consequently we have searched for, and obtained evidence for, a protein kinase responsible for S6 phosphorylation that is more active in transformed cells than it is in their normal counterparts. However this protein kinase appears to be distinct from protein kinase C, the enzyme likely to be activated by the second-messenger diacylglycerol mentioned above, thus other mechanisms for its activation must be entertained. Moreover, our results suggest S6 is a relatively poor substrate for purified protein kinase C. However these data must be considered in view of the capacity of phorbol esters to activate protein kinase C and stimulate S6 phosphorylation in serum-starved cells.

The significance of these various approaches to the functional analyses of $pp60^{v-src}$ will be discussed.

1143 TRANSFORMING GROWTH FACTORS, H.L. Moses, R.F. Tucker and G.D. Shipley, Mayo Clinic, Foundation and Medical School, Rochester, MN 55905

Transforming growth factors (TGF) are generally defined as polypeptides that stimulate normally anchorage-dependent cells to grow in an anchorage-independent manner in soft agar. Since the ability to grow in soft agar is one of the better in vitro correlates of tumorigenicity in certain cell types, TGF's have been proposed as proximal mediators of neoplastic transformation. TGF's are probably also involved in normal growth and development. Two types of TGF's have been well-characterized. TGFα binds to the EGF receptor, has significant sequence homology with epidermal growth factor (EGF), and has biological activities virtually identical to EGF (1,2). TGFβ is very different from TGFα and other well-studied growth factors. We have demonstrated specific cell membrane receptors for TGFβ on a wide variety of cell types, both mesenchymal and epithelial (3). TGFβ is a potent stimulator of growth in soft agar (containing 10% serum) of certain mesenchymal cell types including human foreskin fibroblasts and mouse embryo-derived AKR-2B cells without the requirement for added EGF. TGFβ has been shown to also be a mitogen for the AKR-2B cells in serum-free monolayer culture with a prolonged prereplicative interval relative to other growth factors. TGFβ has different effects on epithelial cells. We have not been able to demonstrate a stimulatory effect of TGFβ on growth of epithelial cells in soft agar. In fact, TGFβ inhibited the spontaneous growth in soft agar of 7 out of 8 carcinoma cell lines (4). TGFβ is also inhibitory for growth of certain epithelial cells in monolayer culture. Collaborative studies between our laboratory and Dr. R.W. Holley have demonstrated that TGFβ is very similar to the growth inhibitor (GI) purified from BSC-1 (African Green monkey kidney) cells (5,6). Both compounds have very similar biological activities and compete for binding to the same cell membrane receptor(s) (6). TGFβ has also been shown to be inhibitory for growth of secondary cultures of normal human epithelial cells in serum-free culture (4). The data demonstrate that TGFβ/GI is a unique growth regulatory polypeptide. It is a potent stimulator of growth in soft agar of certain mesenchymal cells. It is a growth factor for certain mesenchymal cells in serum-free monolayer culture with a prolonged prereplicative interval. It is inhibitory for growth in monolayer and soft agar of a number of epithelial cell types. (Supported by NCI grants CA 16816 and CA 27217.)

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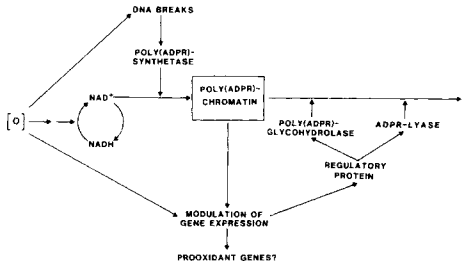
Overview

1144 PROOXIDANT STATES AND PROMOTION, Peter A. Cerutti, Department of Carcinogenesis, Swiss Institute for Cancer Research, Chemin des Boveresses 155, CH-1066 Epalinges, Switzerland

There is convincing evidence that cellular prooxidant states, i.e. increased concentrations of active oxygen, organic-peroxides and -radicals, can promote initiated cells to neoplastic growth. Prooxidant states can be caused by different classes of agents including hyperbaric oxygen, radiation, xenobiotic metabolites and Fenton-type reagents, modulators of the cytochrome P450 electron transport chain, peroxisome proliferators, inhibitors of the antioxidant defence, membrane-active agents. Many of tnese agents cause chromosomal damage by indirect action but the role of this damage in carcinogenesis remains unclear. Prooxidant states can be prevented or suppressed by the enzymes of the cellular antioxidant defence and low molecular weight scavenger molecules. Many antioxidants are antipromoters and anticarcinogens. Prooxidant states may modulate the expression of a family of "prooxidant genes" which are related to cell growth and differentiation by inducing alterations in DNA structure or by epigenetic mechanisms, e.g. by poly ADP-ribosylation of chromosomal proteins (see Figure).

ACTIVE OXYGEN INDUCED MODULATION OF GENE EXPRESSION



P. Cerutti, SCIENCE, in press, and references therein.

Growth Factors and Oncogenes; Tumor Promotion and Progression

1145 A SERINE KINASE ACTIVITY ASSOCIATED WITH p37 env-mos, S. Maxwell and R. Arlinghaus, Dept. of Molecular Biology, Scripps Clinic & Research Foundation, La Jolla, CA 92037

Several isolates of Moloney murine sarcoma virus (Mo-MuSV) encode v-mos gene products that have been found to be associated with a serine protein kinase activity. A temperature-sensitive mutant for transformation (ts110 Mo-MuSV) produces two proteins, termed $P85^{gag-mos}$ and $P58^{gag}$, at the permissive temperature, but only P58 is detectable at non-permissive temperatures. Anti-gag and anti-mos immune complexes, incubated with γ^{32}-ATP and Mn, yield phosphorylated P85. A serine kinase activity is associated with the mos sequences of P85 based on several observations including stringent washing experiments and ts mutant studies [Virology 138: 143 (1984)]. MuSV-124-encoded $p37^{env-mos}$ was also found to undergo phosphorylation in an anti-mos complex yielding not only phosphorylated p37, but also a protein that migrated more slowly on SDS-page (p43). Both in vivo and in vitro phosphorylated p37 generated identical patterns of phosphopeptides when analyzed by the Cleveland technique. Kinetic and structural analyses suggested that p43 is a "super"-phosphorylated form of p37. Antisera specific for the extreme C-terminal sequences of v-mos were inhibitory for the in vitro phosphorylation of both p37 and P85. Phosphoamino acid analyses revealed that both p37 and p43 were being phosphorylated on serine. These observations provide strong evidence that the v-mos sequence encodes a serine protein kinase.

1146 IDENTIFICATION OF A LOW MOLECULAR WEIGHT TRANSFORMING GROWTH FACTOR IN THE URINE OF PREGNANT WOMEN. D.T. Beranek, Natl. Center for Toxicological Research, Jefferson, AR, T.C. Wong and Y.C. Yeh, University of Arkansas for Medical Sciences, Little Rock, AR

Transforming growth factors (TGF) have been linked to the expression of a transformed phenotype in both neoplastic and non-neoplastic mammalian tissues in culture. By using a sequential column chromatography-high pressure liquid chromatography (HPLC) procedure, we have isolated a low molecular weight (M_r<6000) TGF which is present in the urine of pregnant women. Urine from second trimester pregnant females was pooled and eluted through a uBondapak C_{18} Waters Sep-Pak. TGF activity, as measured by an epidermal growth factor (EGF) competition assay, was then eluted from the Sep-Pak with 50% acetonitrile in 0.05% trifluoroacetic acid. After evaporating the acetonitrile under reduced pressure the residue was applied to a Bio-Gel P-30 column, eluted with 100 mM sodium phosphate, pH 7.4, and the fractions containing the low molecular weight TGF-activity were concentrated by elution from a Sep-Pak as above. The recovered activity was then applied to a uBondapak C_{18} reversed-phase HPLC column and eluted with a gradient of 0.05% trifluoroacetic acid in acetonitrile. Five peaks of EGF-competing activity were detected at 20, 30, 35, 39 and 40% acetonitrile. Minor amounts of these peaks were present in the urine from control subjects; however, the peak eluting at 35% acetonitrile was significantly elevated in the urine from pregnant females. In addition to competing with the EGF receptor, this factor also induced [^{3}H]thymidine incorporation in cultured rat kidney cells. These results suggest that TGF's may play a role in normal differentiation and/or human fetal development.

1147 B-LYM EXPRESSION IN HUMAN OSTEOSARCOMA CELLS, P.C. Billings, R.W. Weichselbaum and A.R. Kennedy, Dept. of Cancer Biology, Harvard School of Public Health, Boston, Mass. 02115

We have analyzed B-lym expression in SAOS human osteosarcoma cells. These cells express 5-10 fold higher levels of this gene compared with normal human foreskin fibroblasts. Treatment of thses cells with 1,25 dihydroxy vitamin D, DMSO or retenoic acid had no effect on B-lym expression. Southern blot analysis of genomic DNA from SAOS and normal human foreskin fibroblasts reveals no amplification or rearrangements of B-lym in the SAOS cell line. A comparison of the hybridization patterns using a B-lym probe of Hpa II digested SAOS and normal human DNA reveals the loss of a fragment about 10 kb in size in the SAOS cell line which is present in normal human fibroblast DNA. Both SAOS and normal human cellular DNA give identical hybridization pattens with the isoschizomer Msp I. Our results suggest that a demethylation event, which has occurred in the region of the B-lym gene is responsible for the increased transcription of B-lym in the SAOS cell line.

1148 HUMAN α INTERFERON GENES: DIVERSITY AND EXPRESSION, Arthur P. Bollon, Richard Torczynski and Cheryl Hendrix, Wadley Institutes of Molecular Medicine, Dallas, TX 75235

Human α Interferons contain anti-viral and anti-proliferative properties and have been shown to have anti-cancer activities against select carcinomas. Included in anti-viral activities are recent studies indicating that α Interferon inhibits retroviral proliferation. We have isolated novel α Interferon genes from a human genomic library using 17 base oligomeric probes rather than cDNA probes. The short probes may be useful for identifying subsets of genes of gene families for which cDNA probes either miss or give weak signals. One of the novel α Interferon genes, IFN-αWA, has not been isolated previously, from either cDNA or genomic libraries. IFN-αWA has been sequenced and shown to differ from all previous α Interferon genes at several conserved amino acid positions. IFN-αWA has also been engineered for expression in E. coli using an M13-Lac fusion system indicating that it codes for an active interferon. Another isolated α Interferon gene, IFN-αL, is a pseudogene since it contains a stop codon in the signal sequence. We have corrected the stop codon and have been able to express IFN-αL in E. coli using the M13-Lac fusion system. These studies indicate that the mature interferon sequence has been conserved for active interferon protein. These results bear on the significance of pseudogenes in general, and raise the speculation that the cell may be able to bypass the stop codon. A comparison of IFN-αWA and IFN-L with the other human α Interferons will be presented which may bear on the selective expression of different α interferons as a function of different needs such as anti-viral or anti-proliferation.

1149 A ROLE FOR PROTEIN KINASE C IN CELL PROLIFERATION AND NEOPLASTIC DEVELOPMENT, Alton L. Boynton, Leonard P. Kleine and James F. Whitfield, National Research Council of Canada, Ottawa, CANADA, K1A 0R6.

The Ca^{2+}/phospholipid-dependent protein kinase (PKC) is involved in many types of signal transduction. However its role in mediating the proliferative effects of growth factors, oncogene products and tumor promoting agents is not yet established. We will present evidence suggesting that PKC is involved in cell proliferation and neoplastic development. Non-neoplastic T51B rat liver cells require extracellular Ca^{2+} for their proliferative activity, but their preneoplastic and neoplastic counterparts do not. Thus, non-neoplastic, but not preneoplastic and neoplastic, T51B cells require extracellular Ca^{2+} for the $G_0 \rightarrow G_1$ and $G_1 \rightarrow S$ transitions. Several observations suggest that PKC is involved in these key cell cycle transitions and changes in this enzyme may be involved in neoplastic development. Thus: (1) The PKC activity of non-neoplastic T51B cells is reduced 2-4 fold after proliferative inhibition by Ca^{2+}-deprivation. However, Ca^{2+}-deprivation does not affect PKC activity or proliferation of preneoplastic and enoplastic T51B cells; (2) Two transient surges of PKC activity accompany the Ca^{2+}-dependent $G_0 \rightarrow G_1$ transition and a third more prolonged surge accompanies the Ca^{2+}-dependent $G_1 \rightarrow S$ transition; (3) The tumor promoter and PKC activity or TPA sensitizes T51B cells to extracellular Ca^{2+} which enables them to undergo the $G_0 \rightarrow G_1$ and $G_1 \rightarrow S$ transitions despite extracellular Ca^{2+} deficiencies which would normally prevent them from doing so; (4) Four tumor promoters, TPA, PDD, mezerein and saccharin activate PKC by increasing the affinity of the enzyme for Ca^{2+} and cause non-neoplastic T51B cells to mimic neoplastic cells by proliferating in Ca^{2+}-deficient medium.

1150 RAPID CHANGES IN TRANSCRIPTION INDUCED BY TPA, Timothy H. Carter, Zahra Zakeri-Milovanovic, Mary V. Waldron, and Luis E. Alejo, St. John's University, Jamaica, NY 11439.

The tumor promoter 12-o-tetradecanoyl-phorbol-13-acetate (TPA) activates both cell and virus gene expression. Previous work from our laboratory in collaboration with Paul Fisher at Columbia University has established that TPA decreases the latent period before early virus gene expression can be detected in type 5 adenovirus (Ad5)-infected HeLa cells. This effect is rapid, specific for phorbol esters with tumor promoting activity, independent of new protein synthesis, requires an intact cell, and can be demonstrated by transcription in nuclei isolated from TPA-treated cells (Fisher et. al., Mol. Cell. Biol. 1:370, 1981; Carter et. al., Mol. Cell. Biol.4: 563, 1984). We now report that this TPA-induced change in transcriptional capability can be demonstrated in vitro in a partially purified system containing RNA polymerase II from TPA-treated cells and exogenously-supplied DNA from Ad5 early genes: when template concentration was limiting, TPA-extracts synthesized more "runoff" product than control extracts. The effect was not seen with the Ad5 major late promoter or the LTR sequence from avian sarcoma virus. In vivo TPA suppressed the transcription-delay phenotype of several Ad5 E1a mutants, but this may require some interaction with the E1a gene product.

1151 EXPRESSION OF IMMUNOLOGICALLY REACTIVE αhTGF IN E. COLI. Patrick Clarke, Hermann Herbst, Dennis Slamon, and Martin Cline UCLA, Los Angeles CA 90024

We are studying α human Transforming Growth Factor (hTGF). Current purification methods yield nanogram amounts of hTGF. To improve this yield we are investigating conditions for expressing hTGF in E. coli. We have succeeded in expressing immunologically reactive hTGF fused to the carboxy terminus of the trpE protein. This was achieved as follows: a 175 base pair synthetic gene containing an hTGF coding region was created by sequentially annealing and ligating ten synthetic oligonucleotides. The resulting double stranded fragment was cloned into M13mp9 and sequenced. We tried expressing hTGF alone by subcloning this fragment into the trp promoter vector pRPG49. Crude extracts from cells containing this plasmid, pCLCI, were assayed for hTGF. No hTGF was detectedd by Western analysis or receptor binding assays. We concluded that either no hTGF was being made or that it was being degraded. Generally foreign proteins are more stable in E. coli if they are expressed fused to a bacterial protein. To create a fusion protein we subcloned the fragment into the trpE fusion vector pJLI. The plasmid pHCC37 contained the hTGF coding region in frame at the 3' end of the trpE gene. Western analysis of crude extracts from bacteria containing this plasmid revealed a band of 42 kilodaltons. This band was only present in extracts from cells that had been induced with indolylacrylic acid, and it was absent in cells containing only the parent plasmid. This molecular weight agrees with that predicted for the fusion protein. There are no internal methionines in hTGF. The trpE and hTGF proteins are fused at a methionine. Current efforts are directed at cleaving the trpE::hTGF fusion protein with CnBr and purifying intact hTGF.

1152 PRISTANE AS A PROMOTER OF METHYLCHOLANTHRENE INDUCED LYMPHOID MALIGNANCIES. M.A. Cuchens and L.R. Garrett. Univ. of Miss. Med. Ctr., Jackson, MS 39216-4505

In induction studies wherein 3-methylcholanthrene (MCA) was localized within the Peyer's patch of Copenhagen rats, approximately 5% of the treated rats developed lymphoid malignancies. However if rats were injected with 2,6,10,14-tetramethylpentadecane (pristane) 2 weeks prior to the MCA treatment, B-lymphoid malignancies (leukemias or lymphomas) developed in >50% of the rats. No malignancies developed in rats treated only with pristane. To determine whether pristane induced detectable changes in cellular DNA, flow cytometric analyses were performed. Significant decreases in the relative fluorescent intensity of propidium iodide (PI) stained lymphocytes were observed as early as 4 days post pristane treatment, with a 40% maximum reduction observed 1-2 weeks post treatment. However normal DNA staining was observed by 4 weeks. Furthermore the transient effects of pristane on DNA staining could be reinduced by an additional treatment. Diphenylamine analyses indicated that the shift in PI fluoresence was not due to a quantitative decrease in the amount of DNA. Furthermore no shifts were observed when flow cytometric analyses of PI stained cells were performed at pH 11, rather than pH 7. Collectively these results suggest that pristane may act as a tumor promoter by inducing secondary and/or tertiary changes in nuclear DNA, thereby enhancing the induction of lymphoid malignances by MCA. This investigation was supported by PHS grant number CA33111 awarded by the National Cancer Institute, DHHS.

1153 REGULATION OF EGF RECEPTORS IN HUMAN FIBROBLASTS BY PHORBOL DIESTERS AND PLATELET-DERIVED GROWTH FACTOR, Roger J. Davis, and Michael P. Czech, University of Massachusetts Medical Center, Worcester, MA 01605

The regulation of the EGF receptor was studied in cultured human fibroblasts (WI-38) that had been labeled for 24 hours with [^{32}P] phosphate. Treatment of the cells with 4β-phorbol, 12β-myristate, 13α-acetate (PMA) caused a 3 fold increase in the phosphorylation state of the EGF receptor. Phosphopeptide mapping of the EGF receptor indicated that PMA induced the phosphorylation of the EGF receptor at a site that we have identified as threonine-654 which has been shown to be the site on the EGF receptor that is phosphorylated by C kinase. Platelet derived growth factor (PDGF) was also observed to increase the phosphorylation state of the EGF receptor. Phosphopeptide mapping demonstrated that PDGF induced the phosphorylation of the EGF receptor at the same site that is phosphorylated in the presence of PMA (threonine-654). This specific phosphorylation of the EGF receptor at threonine-654 may be the mechanism by which these agents regulate the apparent affinity of the EGF receptor in fibroblasts. These results suggest that the rise in the level of diacylglycerol and Ca^{++} caused by PDGF stimulates the activity of C kinase and that this enzyme is involved in the mitogenic response of cells to PDGF.

1154 INTERLEUKIN-2 RECEPTORS ON ANTIGEN-SPECIFIC HUMAN T CELL LINES: MODULATION BY ANTIGEN ACTIVATION AND IL-2. Elaine C. DeFreitas, The Wistar Institute, 36th & Spruce Streets, Philadelphia, PA 19104

Continuous human T helper cell lines specific for tetanus toxoid (Tet) and purified protein derivative (PPD) require repeated antigenic stimulation to resume proliferation in Il-2. These lines were examined for their expression of the Il-2 receptor using the monoclonal antibody anti-Tac [Leonard et al. (1982) Nature 300:207]. The cells show a transitory expression of Il-2 receptors which reach a maximum level three days after presentation of specific antigen on autologous monocytes. T cell proliferation preceeded the maximal appearance of Il-2 receptors by 2 days. When the T cell lines are resuspended in saturating concentrations of recombinant Il-2, Tac expression reaches a maximum after 2 days then decreases to reach background levels by day 10-11. Monoclonal antibody against HLA-DR antigen abrogates the proliferation of T cells in response to antigen but does not affect replication of cells in Il-2. These data show that induction of Il-2 receptors by T cells is dependent on specific antigen recognition but maintenance of those receptors does not depend on continual antigenic stimulation. In addition, down-regulation of Il-2 receptors occurs in the presence of saturating concentrations of Il-2 indicating a basis for normal control of T cell proliferation.

1155 CHARACTERIZATION OF SOME cDNA CLONES OF MAMMALIAN mRNA SPECIES EXPRESSED IN A CELL-CYCLE-DEPENDENT MANNER, David T. Denhardt, Craig L.J. Parfett and Dylan R. Edwards, Cancer Research Laboratory, University of Western Ontario, London, N6A 5B7 Canada.

A cDNA library constructed in pBR322 from poly(A)mRNA isolated from cultured mouse embryo fibroblasts was enriched for low abundance species and then screened for clones corresponding to cytoplasmic mRNAs that showed a variation in levels of expression as cells proceeded out of the quiescent Go phase, through G1, and into S phase. A subset of the clones identified were observed to correspond to mRNAs whose abundance relative to the major mRNA species actually decreased as the serum-stimulated cells proceeded through G1; all but one of these were found to be clones of mitochondrial mRNAs (Edwards & Denhardt, Expt. Cell Res., in press). The remaining clones correspond to mRNAs that increase in abundance in the cytoplasm as the cells proceed towards S phase. For some clones the level remains high, but in at least one case the level is maximal in G1. Two of the "serum-induced" mRNAs were found at equal levels in nuclei from quiescent and serum-stimulated cells suggesting that increased mRNA levels in the cytoplasm is the result of enhanced processing/transport rather that increased transcription of the nuclear genes. (Supported by the NCI and MRC of Canada.)

1156 STRUCTURE OF HUMAN TRANSFORMING GROWTH FACTOR -α AND -β AND THEIR PRECURSORS, Rik Derynck, Ellson Chen, Dennis Eaton, Julie Jarrett, Ann Van Tilburg, John Bell and David V. Goeddel, Genentech, Inc., South San Francisco, CA 94080; Richard Assoian, Anita Roberts and Michael Sporn, National Institutes of Health, Bethesda, MD 20205

Transforming growth factors (TGFs) can induce anchorage-independent growth of normal cells in soft agar. TGF-α competes with epidermal growth factor (EGF) for the same receptor and is synthesized by a variety of tumor cells. TGF-β, a homodimeric protein, is secreted by many normal and tumor cells and binds to a different receptor. TGF-α and TGF-β coordinately induce the anchorage-independent growth of NRK cells. However, TGF-β also has a growth inhibitory activity on many cells. We have isolated cDNAs and genes for both TGF-α and TGF-β. The deduced amino acid sequences show that TGF-α is made as part of a 160 amino acid long precursor which is encoded by 4.8 kb mRNA. The TGF-α gene is comprised of 6 exons which are spread over at least 70 kbp. Sequence analysis of TGF-β cDNAs shows that the TGF-β monomeric polypeptide is 112 amino acids long, contains 9 cysteines, and that it is encoded as part of a large hydrophobic precursor. TGF-α and TGF-α cDNAs have been used to probe for the presence of the mRNAs in a variety of human tissues, tumors and tumor cell lines.

1157 TRANSFORMING GENE PRODUCT OF SIMIAN SARCOMA VIRUS, A PROTEIN CLOSELY RELATED TO HUMAN PLATELET DERIVED GROWTH FACTOR, S. Devare, J. Casey,C. Scheffel, D. Norton, P. Andersen and C. Wood*, Departments of Molecular Biology and Cancer Research*, Abbott Laboratories, North Chicago, Illinois 60064.

Comparison of predicted amino acid sequence of simian sarcoma virus (SSV) transforming gene product deduced from nucleotide sequence of the viral genome with the partial amino acid sequence of several peptides from human platelet derived growth factor (PDGF) demonstrates that these two proteins have arisen from closely related cellular genes. Sequence homology between these two biologically important proteins has provided a unique means for immunological and structural characterization of SSV transforming gene product as well as PDGF. We have raised antibodies to various domains of proteins in the regions of close amino acid sequence homology between these two proteins and also in regions of SSV transforming gene product where no sequence homology has been demonstrated with PDGF. Data will be presented on characterization of these antibodies and their ability to recognize these two proteins.

1158 TRANSFORMING GENES FROM BENZO(A)PYRENE-TRANSFORMED SYRIAN HAMSTER CELL LINES. KATHLEEN PIROLLO, NEBOJSA AVDALOVIC, and LEILA DIAMOND. The Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104.
B(a)P-transformed, tumorigenic Syrian hamster embryo and epidermal cell lines were examined for the presence of activated ras genes by transfection of genomic DNA into NIH/3T3 cells. The DNA from two cell lines produced transformed foci at a frequency of 0.015 foci/μg DNA; the transforming frequencies were 2-3-fold higher with DNA from primary and secondary transfectants. The 3T3 transfectants of both cell lines produced tumors when inoculated s.c. into nude mice; most tumors appeared 10-14 days after inoculating 10^6 cells. The transforming ability of the DNA from the embryonic fibroblast cell line, HE68, was lost after cleavage with EcoRI, whereas that of DNA from the epidermal cell line, JH-11, was not. High stringency hybridization of v-ras^H (BS-9) and v-ras^K (HiHi380) probes to PstI-restricted DNA from both normal and transformed hamster cells gave bands at 5.0 and 2.1 Kb and 9.7, 8.0, 5.0 and 4.0 Kb, respectively. Under low stringency hybridization conditions, the intensity of hybridization of DNA from primary 3T3(HE68) transfectants to a ras probe was greatly increased in the high MW range but, under high stringency conditions, the transfectant DNA showed no hamster-specific bands with either the BS-9 or HiHi380 probe. The DNA from primary 3T3(JH-11) transfectants produced no hamster-specific bands with these probes under low or high stringency conditions. Thus, the two B(a)P-transformed hamster cell lines contain different transforming genes, one of which appears to be an activated ras gene and the other is as yet unidentified.

1159 IDENTIFICATION OF GROWTH FACTORS FROM CULTURED HUMAN BREAST CANCER CELLS, Robert B. Dickson, Karen K. Huff, Susan Bates and Marc E. Lippman, National Cancer Institute, NIH, Bethesda, MD 20205. Human breast cancer cell lines in serum free medium secrete autocrine mitogenic activities as well as transforming growth factors (TGF) for fibroblasts. Fractionation of conditioned medium on Sephacryl S-200 chromatography demonstrated that these two classes of mitogenic activities were heterogeneous, only partially overlapping families of polypeptides. Estrogen treatment of an estrogen-receptor containing cell line (MCF-7) induced autocrine mitogens 2-3-fold and TGF activity up to 8-fold. Estrogen receptor containing (MCF-7,ZR-75-1) as well as receptor negative lines (Hs578T, MDA-MB-231) also produced detectible EGF-receptor competing polypeptides suggestive of TGFα activity. EGF-receptor competing polypeptides were estrogen induced 4-6-fold in MCF-7 cells. These polypeptides were heterogeneous upon acid-Biogel P60 analysis. In addition, all four cell lines contained and secreted immunoreactive IGF-1. In MCF-7 cells, IGF-1 appeared to be a high molecular weight species (55 KDa) which could be converted to a lower molecular weight species following acid/ethanol extraction. This IGF-1 like species was similar to authentic serum-derived IGF-1 in radioimmunoassay displacement curve profile and apparent molecular weight. IGF-1-like and EGF-related polypeptides may act together as autocrine or paracrine tumor growth factors in human breast cancer.

1160 STABLE INTEGRATION OF MUTANT c-Ha-ras ONCOGENE INTO C3H/10T1/2 CELLS AND ITS RELATIONSHIP TO TUMORIGENIC TRANSFORMATION, William E. Fahl, T. Herbert Manoharan, Jeffrey A. Burgess and Dominic Ho, Northwestern University Cancer Center, Chicago, IL. 60611

C3H/10T1/2-CL8 mouse cells were shown to take up and express a plasmid-cloned drug resistance gene (Ecogpt) after DNA transfection at a frequency (2-6 X 10^{-5}) which is acceptable for routine recovery of gene-transformed populations. Transfection of 10T1/2 cells with a mutant c-Ha-ras oncogene isolated from the human EJ bladder tumor cell line (pEJ6.6 plasmid) results in neoplastically transformed 10T1/2 cell populations as judged by colony morphology and tumorigenic growth in nude mice. The levels of mutant c-Ha-ras gene integration and expression in the tumorigenic cell populations and 10T1/2 cell controls were determined, and the highest level of mutant ras transcript was seen in the most tumorigenic cell population. A preliminary comparison of 10T1/2 and NIH/3T3 cells showed similar frequencies for pEJ6.6 induced transformed foci and similar sensitivity to Ha-ras and B-lym transforming effects. The results indicate a carcinogen-inducible genetic event which is permissive for neoplastic transformation of this widely used, carcinogen-transformable mouse cell line.
Supported by NIH grants CA-25189 and CA-35514.

1161 MEMBRANE SIGNAL TRANSDUCTION BY INTERLEUKIN 2 FOR THE INITIATION OF GAMMA INTERFERON mRNA SYNTHESIS. William L. Farrar and Howard A. Young, Laboratory of Molecular Immunoregulation, National Cancer Institute-FCRF, Frederick, MD. 21701

Interleukin 2 (IL-2) is a lymphocytotropic growth peptide for T lymphocytes and large granulocyte lymphocytes. In addition to the promotion of S phase progression, IL-2 has been shown to induce the release of gamma interferon(IFN-γ)from lymphocytes(Farrar, Johnson and Farrar, J. Immunol. 1980). Utilizing a cell line, BUD-27, derived from an IL-2 dependent murine T lymphocyte clone(CT6), we have demonstrated that recombinant human IL-2 stimulates mRNA synthesis of murine IFN-γ as determined by cDNA hybridization. Additionally, phorbol myristate acetate(PMA), the diacylglycerol derivative 1-oleyl-2-acetyl-glycerol(OAG), and the calcium ionophore A23187, also stimulated IFN-γ mRNA synthesis in cultured BUD-27 cells.Stimulation of IFN-γ mRNA synthesis in vivo by Protein Kinase C(PK-C) activators(PMA,OAG) suggested that IL-2 may induce IFN-γ mRNA synthesis through a mechanism involving PK-C activation. BUD-27 cells were stimulated with IL-2 and other stimulants of IFN-γ mRNA synthesis and the PK-C enzyme levels in cytosol and plasma membranes quantitated. IL-2 stimulated a ten-fold increase in PK-C activity associated with the plasma membranes of BUD-27 cells. Although a concomitant decrease in cytosolic PK-C activity was observed, no change in total cellular PK-C activity was seen. Similar patterns of PK-C subcellular redistribution was also observed with PMA and A23187. The induction of IFN-γ production by IL-2 showed a dose-dependent correlation between PK-C membrane association and IFN-γ yields. These data suggest a correlation between PK-C activation by IL-2 and signal transduction for the expression of inducible genes for secretory proteins derived from lymphocytes.

1162 CHEMILUMINESCENCE IN MOUSE EPIDERMAL CELLS INDUCED BY TPA AND PHOSPHOLIPASE C. S.M. Fischer and L.M. Adams. Univ. Texas System Cancer Center, Science Park-Research Division, Smithville, TX.

The increased production of oxidant species has been suggested to be a component of the cellular response to the tumor promoter 12-O-tetradecanoylphorbol acetate (TPA). We have demonstrated this in mouse epidermal cells using a luminol-dependent chemiluminescence (CL) assay. This assay uses 10^7 cells, isolated from newborn SENCAR mice in phosphate buffered saline containing 1 μg/ml luminol, a CL enhancer. The CL response to TPA is dose- and cell-number dependent. Inhibitors of various parts of the arachidonic acid cascade were found to affect the TPA-induced CL response in a manner that corresponds to their effects in in vivo tumor promotion experiments: agents which inhibit lipoxygenase, ie, NDGA, benoxaprofen, ETYA, and phenidone, are much more effective in diminishing the CL response than cyclooxygenase inhibitors, ie, indomethacin and flurbiprofen. This suggests that a major part of the CL response is due to arachidonate metabolism by the lipoxygenases. Additionally, phospholipase C (PLC) but not phospholipase A_2 or D induced a dose-dependent CL response kinetically similar to the TPA response. A common pathway, phosphatidylinositol turnover-protein kinase c, may exist for the TPA and PLC response. A comparison of several strains of mice showed that their sensitivity to TPA as a promoter in vivo correlates with the magnitude of the CL peak induced by either TPA or PLC, further suggesting that the oxidant species produced is involved in the mechanism of tumor promotion.

1163 COPPER IONS AND HYDROGEN PEROXIDE FORM HYPOCHLORITE FROM NaCl MIMICKING MYELOPEROXIDASE, Krystyna Frenkel, Frederic Blum and Walter Troll, New York University Medical Center, New York, NY 10016.

Oxygen radicals have been implicated as mutagens and carcinogens. Active oxygen species include superoxide anion radical ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical (·OH) and hypochlorite (OCl^-). Superoxide dismutase (SOD) and SOD-mimetic compounds such as cupric diisopropyl salicylate (CuDIPS) inhibit tumor promotion. CuDIPS and $CuCl_2$ both block fertilization of the sea urchin Arbacia punctulata. It was thought that the SOD-mimetic activity of Cu^{2+} generating H_2O_2 was responsible for the inactivation of the sperm. Surprisingly, instead of increasing the level of H_2O_2, Cu^{2+} decreased it. The product(s) of the interaction of Cu^{2+} with H_2O_2 was 10^3 fold more active in the inactivation of the sperm than H_2O_2 alone. This increased activity was thought to be caused by ·OH which was presumably formed in a similar manner to that of Fe^{2+}/H_2O_2 system. However, this toxic product was not ·OH because Fe^{2+} did not block fertilization and the activity of Cu^{2+} was not counteracted by ·OH scavengers mannitol and thiourea. In contrast, the OCl^- scavenger 2-aminotriazole completely inhibited the toxic action of Cu^{2+}. Furthermore, OCl^- was generated by solutions of H_2O_2, Cu^{2+} and seawater containing 1M Cl^-. Thus, it is possible that the antifertility properties of Cu^{2+} are due to its ability to mediate formation of OCl^-. Since sperms contain myeloperoxidase, an enzyme known to catalyze oxidation of Cl^- by H_2O_2, it may be that OCl^- is the final product elaborated by the sea urchins to avoid polyspermy. Furthermore, OCl^- may play a role in the oxidative and mutagenic properties of H_2O_2 in the presence of Cu^{2+}. (Supported by PHS Grant No. CA 16060, awarded by the NCI, DHHS).

1164 THE NATURE OF THE ANTITRANSFORMING ACTION OF RETINOIC ACID IN MOUSE EPIDERMAL CELLS, Helen L. Gensler, Lynn M. Matrisian, and G. Tim Bowden, University of Arizona, Tucson, AZ 85724

JB6 mouse epidermal cells are induced to anchorage independent growth by epidermal growth factor (EGF) as well as by late stage tumor promoters. β-all-trans-retinoic acid (RA) inhibited EGF-induced transformation in a dose dependent manner. Mezerein-induced transformation was inhibited to a greater extent by RA than was EGF-induced growth in soft agar, at similar colony yields. Anchorage dependent growth was inhibited by RA to a similar extent whether the RA was applied before or after transformation induced by EGF. Therefore, transformation per se did not change the sensitivity of the cells to retinoid inhibition of anchorage dependent growth. The extent of retinoic acid induced inhibition of growth in soft agar was as great when retinoic acid was applied after EGF or TPA induced transformation, as when it was applied during promoter induced transformation. Thus, the anti-proliferative action of RA, without an additional antitransformation effect, was sufficient to account for the reduced number of colonies formed in soft agar in the presence of RA.

1165 IN VITRO MUTAGENESIS OF V-SIS IDENTIFIES REGIONS OF THE GENES THAT ARE NECESSARY FOR CELLULAR TRANSFORMATION, Neill A. Giese, C. Richter King, Keith C. Robbins and Stuart A. Aaronson, National Institutes of Health, National Cancer Institute, Bethesda, MD 20205

The transforming gene, v-sis, of simian sarcoma virus codes for a 28 kd protein, p28sis, which is highly homologous to human platelet-derived growth factor (PDGF). In transformed cells this protein dimerizes and then undergoes proteolytic cleavage at both the carboxy and amino termini. Mutants of v-sis which code for proteins that lack amino acid sequences at either end of the molecule were analyzed for transforming activity and protein structure. These studies demonstrate that coding regions of the v-sis gene that lie outside of the known homology to PDGF are not necessary for transforming activity.

1166 AMPLIFICATION OF VIRAL ONCOGENE SEQUENCES CORRELATES WITH THE TUMORIGENIC PHENOTYPE IN SYRIAN HAMSTER EMBRYO CELL LINES, Tona M. Gilmer, Pattie Lamb, Lois Annab, Mitsuo Oshimura and J. Carl Barrett, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

Gene amplification of cellular oncogenes has been implicated in the development of cancer in humans. We have examined gene dosage effects following transfection of preneoplastic Syrian hamster embryo (SHE) cell lines with a genomic clone of Rous sarcoma virus (RSV) which encodes the v-src gene. The 10W cell line, isolated after treatment of SHE cells with asbestos, was co-transfected with pSV2-neo and RSV DNAs and selected for antibiotic (G418) resistance (neo^R). None of 12 neo^R clones isolated after co-transfection expressed v-src RNA; however, three clones (clones 41,61 and 62) contained multiple copies of RSV DNA. Upon injection into nude mice, clones 41, 61 and 62 were tumorigenic with short latency periods of 2-4 weeks, while none of 10 neo^R clones isolated following transfection of 10W cells with pSV2-neo DNA alone were tumorigenic. Tumor-derived cell lines from clones 41, 61 and 62 expressed high levels of v-src RNA and had an approximately 10 to 20-fold increase in RSV DNA sequences, which appeared to be located in double minute chromosomes. The mechanism of suppression of RSV DNA sequences (i.e., state of methylation, cellular factors) in these clones and the release of that suppression by gene amplification are under investigation. These results will be compared with the effects of other viral oncogenes on the 10W cells.

1167 ISOLATION AND CHARACTERIZATION OF AMPLIFIED DNA SEQUENCES FROM MULTI-DRUG RESISTANT HAMSTER CELLS, Philippe Gros, James M. Croop, Igor Roninson, David Housman, Massachusetts Institute of Technology, Center for Cancer Research, Department of Biology, Cambridge, MA 02139

We have cloned and anlayzed over 100 kilobases (kb) of contiguously amplified DNA from two independently derived multi-drug resistant cell lines (LZ cell, HOWELL et al, 1983; C5 cell, LING et al, 1981). Using the technique of in-gel renaturation (Roninson et al, 1983) a series of Bam H-1 DNA fragments were identified and found to be amplified in both LZ and C5 cells. Via a modification of the original in-gel renaturation procedure a 1-1kb fragment was cloned. This clone was then used as a hybridization probe to initiate the isolation of the entire amplification unit in cosmid vectors. We report here the isolation of over 100 kb of amplified DNA from the LZ cell line. Using in gel renaturation between cloned DNA and genomic DNA from the drug resistant cell lines, we have demonstrated colinearity of cloned restriction fragments and amplified DNA sequences. Analysis of transcription from the amplified DNA region indicates that an m RNA species of 6kb is encoded by the amplified region. The level of expression of this m RNA is correlated with the level of amplification and the degree of drug resistance. These results suggest that the 6kb m RNA plays a functional role in multi-drug resistance. Genomic DNA sequences homologous to the 6kb m RNA span over 80kb of the amplified DNA segment.

1168 MODULATION OF MOUSE MAMMARY TUMOR VIRUS (MMTV) EXPRESSION BY DEXAMETHASONE, TPA, AND ANTIOXIDANTS, D.C. Gruenert*, J. Friedman, and P.A. Cerutti, Institut Suisse de Recherches Experimentales sur le Cancer, 1066 Epalinges/Lausanne, Suisse
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Tumor promotion and glucocortical hormone action is accompanied by changes in gene expression and the induction of oncogenic viruses. 12-O-tetradecanoyl-phorbol-13-acetate (TPA) is a potent tumor promoter whose mode of action appears to be mediated by the formation of oxygen radicals. TPA also activates a specific cell membrane receptor with protein kinase activity. We have examined the modulation of TPA and dexamethasone-induced MMTV production by antioxidants. By using RMA blot hybridization, we assessed the effect of superoxide dismutase and catalase on the induction of MMTV by TPA and dexamethasone. Superoxide dismutase and catalase have very little effect on the MMTV induction by dexamethasone. TPA induction of MMTV is inhibited by catalase, but not by superoxide dismutase. We also detected a slight, but reproducible stimulation of MMTV by H_2O_2.

1169 Synthesis, Cloning and Expression in E.coli of Genes Coding for TGFα, David L. Hare+, Margery A. Nicolson*, Marie A. Cecchini+, Zipora Stabinsky+, Mary D.M. Carter+, Julia K. Tseng*, and Allen R. Banks+, +Amgen Development Incorporated Boulder Colorado 80301, *Amgen Incorporated Thousand Oaks California 91320.

Transforming growth factor alpha (TGFα) is a 50 amino acid polypeptide that has been correlated with neoplastic transformation because it is found only in the conditioned medium of transformed cell lines. TGFα has in vitro properties nearly identical to those of epidermal growth factor. For example, TGFα competes with ^{125}I-labeled mouse EGF for receptors on human A431 cells and both proteins induce NRK cells to form colonies in soft agar. These corresponding in vitro properties suggest that TGFα and EGF have homologous amino acid sequences, however, TGFα has less than 30% sequence homology with EGF.

TGFα has been isolated from the conditioned medium of transformed cell lines, but only small quantities of the protein can be obtained in this manner. In order to obtain enough material to study the biochemical action of TGFα and TGFα analogs, we have constructed synthetic genes which code for both human TGFα and rat TGFα and we have expressed both genes at high levels in E.coli. These recombinant DNA-derived proteins have the in vitro biological properties expected for TGFα. Internal restriction sites were included in the TGF gene constructions which have allowed the expression of TGF-EGF hybrid genes at high levels in E.coli as well. We anticipate that TGFα and analogs generated using synthetic gene technology will assist in assigning functional domains within the protein to corresponding identified biological properties of TGFα.

1170 THE STRESS RESPONSE OF HUMAN PRIMARY FIBROBLASTS AND ITS POSSIBLE IMPLICATIONS FOR CARCINOGENESIS, Peter Herrlich, Peter Angel, Hans Rahmsdorf, Christine Lücke-Huhle, Marina Schorpp and Annette Pöting, Kernforschungszentrum Karlsruhe, Institut für Genetik und Toxikologie, Postfach 3640, D-7500 Karlsruhe 1, Fed. Rep. of Germany

Inherited cancer has provided a hypothesis of carcinogenesis involving a frequent and a rare event. In induced cancerogenesis, a frequent step is also likely to occur which must be due to the mutagenic treatment, and which is yet ill-defined. We have detected and characterized a stress response of mammalian cells which may serve as a candidate for such an early step in carcinogenesis. Using cDNA and genomic cloning techniques we have isolated sequences which are more abundantly expressed after treatment of cells with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). The set of responsive genes turned out to be part of a generalized stress response in that treatment of cells with ultraviolet light and mitomycin C would lead to the same, serum starvation to partly the same response. Responsive genes sequences and functions include the cellular fos oncogene, the metallothionein IIA gene, a trans acting function that causes gene amplification, and a secreted factor which transfers the response through the extracellular medium to cells that had not been treated at all. We have detected one high-cancer-incidence disease which expresses the "stress genes" constitutively: Bloom Syndrome. This suggests that the stress response leads to increased rate of mutagenesis. Metallothionein may serve as a general radical scavenger since cells with high expression seem to survive better upon gamma irradiation. The stress response may thus - as in bacteria - include protective and mutagenic functions.

1171 MOLECULAR CLONING OF THE NEU ONCOGENE, Mien-Chie Hung, Alan L. Schechter, Lalitha Vaidyanathan and Robert A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, MA 02142

The neu- oncogene is a transforming gene that was found in the genomes of several rat neuro/glioblastoma cell lines. The neu gene was shown to be related to erbB oncogene and reside in its entireity on a 33Kb ECoRI segment of DNA in the rat genome. To isolate the biologically active version of neu, a cosmid library was constructed by using ECoRI digested DNA from a neuroblastoma transfectant in which the rat neu gene was amplified in the mouse NIH3T3 cells. This library was screened by using erbB as a probe and several recombinant clones containing erbB homologous sequence were isolated. Subsequently, structural analysis by Southern blotting indicated one of the recombinant clones contained restriction enzyme patterns specific to the neu gene. Transfection of this clone into NIH3T3 cells resulted in the appearance of transformed cells on the monolayer. These foci were shown to produce the novel 185,000 dalton tumor antigen which was originally found to be associated with the neuro/gliblastomas. These results indicate that an biologically active neu gene is molecularly cloned.

1172 STOICHIOMETRIC BINDING OF DIACYLGLYCEROL TO THE PHORBOL ESTER RECEPTOR / PROTEIN KINASE C. Bernhard König and Peter M. Blumberg, Molecular Mechanisms of Tumor Promotion Section, Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205

The phorbol esters represent the most potent class of tumor promotors for mouse skin. In addition to their tumor promoting ability, they exert profound effects on a wide variety of other cellular systems. The initial event in the mechanism of action of the phorbol esters appears to be their binding to a high affinity receptor which represents a complex between specific phospholipids, Ca^{2+}, and the apo-enzyme protein kinase C. This complex is believed to play an important role in transducing the signals of extracellular messengers whose earliest effects are associated with enhanced breakdown of phosphatidylinositol-4,5-bisphosphate. Diacylglycerol, one product of this breakdown, stimulates protein kinase C in a fashion similar to phorbol esters. Since diacylglycerol, the postulated endogenous activator of protein kinase C, inhibits phorbol ester binding competitively, we suggested that diacylglycerol is the/an endogenous phorbol ester analog. Therefore, one would predict that diacylglycerol binds to protein kinase C with 1:1 stoichiometry (as the phorbol esters do) and interacts at the same site on the enzyme. The alternative is that diacylglycerol is simply acting by modifying the phospholipid environment of the protein. We present evidence, that, as predicted for an endogenous analog, diacylglycerol inhibits phorbol ester binding stoichiometrically.

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UNIQUE ALLELIC RESTRICTION FRAGMENTS OF THE HUMAN HA-ras LOCUS IN TUMOR AND LEUKOCYTE DNA OF CANCER PATIENTS. Theodore G. Krontiris, Nancy A. DiMartino, Mark Colb and David R. Parkinson, Tufts Cancer Research Center, Boston, MA 02111

The highly polymorphic human Ha-ras locus may be quite useful as a marker for inherited susceptibility to cancer. Twenty-four allelic restriction fragments have thus far been detected by Southern blotting of white blood cell (WBC) and tumor DNAs of unrelated Caucasians. Fifteen of the twenty-four alleles have only been detected in WBC or tumor DNA of cancer patients ($p<0.001$). Family studies and analysis of matched tumor/WBC DNAs indicate that these rare alleles are inherited in a Mendelian fashion. In some subsets of patients there is a particularly high rate of occurrence of unusual alleles. For example, in the pre-leukemic state called myelodysplasia, 6 of 9 patients possessed rare alleles (compared to 25% of the entire cancer patient population). One of these alleles, a2.2, was detected in two myelodysplastics, two acute leukemics and a patient with essential thrombocythemia (increased platelet production). Half of patients with melanoma (ninety per cent of those with familial melanoma) possess rare Ha-ras alleles. These results suggest that (a) particular Ha-ras alleles may be associated with increased risk for certain forms of cancer; (b) Ha-ras may play a more prominent role in human tumorigenesis than data from DNA transfections suggest; and (c) such a role is not tissue-specific, since all forms of cancer demonstrate these alleles. We have now begun to characterize molecular clones of several rare allelic fragments from patients with myelodyplasia and with melanoma in an attempt to determine if germline abnormalities of the Ha-ras gene may be detected.

1174 BIOSYNTHESIS OF THE V-SIS/PDGF-2 TRANSFORMING GENE PRODUCT, Fernando Leal, Jacalyn Pierce, Keith C. Robbins and Stuart A. Aaronson, National Institutes of Health, National Cancer Institute, Bethesda, MD 20205

Recent evidence that the acute retrovirus transforming genes v-sis and v-erb B encode proteins bearing striking homology to platelet-derived growth factor (PDGF) and the epidermal growth factor receptor, respectively, has implicated those pathways which control normal cellular proliferation in the process leading to transformation. As one approach toward understanding the mechanism by which simian sarcoma virus (SSV) induces morphologic transformation, we have described the biosynthetic pathway of the SSV transforming gene product, a molecular related to human PDGF polypeptides chain 2. Our findings reveal that the primary v-sis translational product, $p28^{sis}$, is a glycoprotein synthesized on membrane bound polyribosomes. After its synthesis, the molecule dimerizes in the endoplasmic reticulum and travels toward the periphery of the cell where it is further processed to yield a polypeptide structurally analogous to biologically active PDGF. Small amounts of two incompletely processed forms are selectively released into the culture medium of SSV transformants. However, the vast majority remains cell associated. At steady state, most of the sis proteins are bound to internal cell membrane components, while approximately 10% is exposed on the cell surface. These results define the cellular locations where the transforming activity of this protein may be exerted.

1175 PROGRESSION OF THE TRANSFORMED PHENOTYPE IN TYPE 5 ADENOVIRUS (Ad5) TRANSFORMED RAT EMBRYO CELLS IS A REVERSIBLE PROCESS, W.S. Liaw[1], L.E. Babiss[1], S.G. Zimmer[2] and P.B. Fisher[1], Columbia Univ.[1], College of Physicians & Surgeons, New York, NY 10032 and Univ. of Kentucky Medical Ctr.[2], Lexington, KY 40536.

The carcinogenic process is multifactor in terms of its etiology and multistep in its development. Based on in vivo studies the carcinogenic process has been divided into three (sometimes overlapping) phases, termed initiation, promotion and progression. Infection of early passage rat embryo cells with type 5 adenovirus (Ad5) can result in morphologically transformed cells which display only some of the phenotypes often associated with viral transformation. Continuous passage of transformed cells in monolayer culture, growth of transformed cells in agar containing or lacking the tumor promoting agent TPA or tumor induction in nude mice results in subclones which display an enhanced expression of the transformed phenotype. Progression does not involve a change in the location or quantity of integrated Ad5-DNA sequences, but is associated with an increase in methylation of sequences other than those of the transforming E1 region. A single treatment of highly progressed Ad5-transformed clones with the demethylating agent 5-azacytidine (Aza) results in transformed subclones which exhibit an early stage in transformation. Reisolation of Aza-treated clones following growth in agar results in reaquisition of the progressed phenotype. These observations indicate that progression of the transformed phenotype can either be accelerated or reversed depending on the type of manipulation employed. These cell culture systems should, therefore, prove valuable in defining the role of cellular and viral genes in regulating expression of the transformed state. (Supported by CA 35675).

1176 LYSOSOMOTROPIC AMINES DO NOT INHIBIT ABILITY OF EGF OR TPA TO INDUCE VL30 GENE ACTIVITY, B. E. Magun, Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR 97201

The cellular mechanisms by which EGF and its receptor are able to transmit signals in the activation of cellular genes are largely unknown. The laboratory of M. Getz has previously reported that EGF was able to induce transcripts of VL30 RNA in mouse AKR-2B cells (PNAS 79: 7317, 1982). VL30 RNA are 30S molecules with retrovirus-like properties. Presence of VL30 RNA was detected by Northern blotting of total RNA followed by hybridization to a nick-translated VL30 plasmid (provided by M. Getz). Upon addition of EGF to AKR/2B cells, VL30 RNA was induced several fold and was maximal between 3 and 6 h. VL30 RNA levels returned almost to baseline levels by 12 h. The same time course of induction and decline was observed following treatment with the phorbol ester TPA, except that several fold more VL30 was induced by the phorbol ester than by EGF. TPA, but not EGF, induced VL30 genes in EGF-repectacles NR6/3T3 cells (provided by H. Herschman). Cycloheximide not only failed to block EGF- or TPA-stimulated VL30 induction, but apparently caused a superinduction, perhaps by inhibiting VL30 degradation. Furthermore, a higher MW form of VL30 appeared, perhaps representing a precursor. The lysosomotropic amine methylamine, which inhibits processing and degradation of EFG and its receptor within cytoplasmic vesicles, had no observable effect on induction of VL30 activity. We propose that VL30 induction is an early event induced by a signal generated in the absence of vesicular processing, and that protein kinase C activation by EGF or TPA may be involved in transmission of the signal.

1177 THE ISOLATION, SEQUENCING, AND CHARACTERIZATION OF A NEW HUMAN PROTO-ONCOGENE RELATED TO THE RAF ONCOGENE, George E. Mark and Todd W. Seeley, Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD 20205.

The raf oncogene is an evolutionarily ancient member of the largest family of oncogenes, the src family. Two raf homologs have been identified in Drosophila, and cloned. It, therefore, seemed likely that two homologs would exist in man. Screening of a human fetal liver cDNA library with the murine v-raf oncogene probe revealed two related sequences. One corresponds to the previously described human c-raf-1 locus, while the other was unique as judged by restriction enzyme analysis. Southern analysis of human DNA with probes specific to these cDNAs also attested to the individuality of each sequence. Comparison of this second sequence, to be called c-raf-3, by DNA heteroduplex to c-raf-1 revealed homolog only in the kinase domain. The DNA sequence, chromosomal localization, and the expression of this new proto-oncogene will be presented.

1178 STRUCTURAL PROPERTIES OF RECEPTORS FOR TYPE β TRANSFORMING GROWTH FACTOR. Joan Massague, Betsy Like and Brenda Kelly. University of Massachusetts, Worcester, MA, 01605.

Type β transforming growth factor (βTGF), a dimeric, disulfide-linked polypeptide, binds with high affinity and specificity to receptors in target cells. Cellular components with the characteristics of affinity and specificity expected from physiologically relevant βTGF receptors have been affinity-labeled in human, rodent, and chick cells and membrane preparations using ^{125}I-βTGF and disuccinimidyl suberate. βTGF receptors affinity-labeled in mouse, rat and chick cells migrate as 280 kilodalton species when electrophoresed in the presence of dodecyl sulfate and reductant. βTGF receptors in human fibroblasts exhibit larger apparent Mr, but comparative peptide maps indicate strong homology with βTGF receptors from rodent cells. In the absence of reductants βTGF receptors migrate as large complexes in electrophoretic gels. βTGF receptors have been solubilized in the presence of non-ionic detergents under conditions in which the ligand binding and affinity-labeling properties of the native state are preserved. The hydrodynamic properties of βTGF receptors in the native state are consistent with a molecular size of about 550 kilodalton. βTGF receptors from all sources examined bind to, and can be specifically eluted from immobilized lectin columns. These results suggest that the membrane receptor for βTGF is an oligomeric glycoprotein with a 280 kilodalton βTGF binding subunit linked to other subunits via disulfide bonds. It is possible that this complex is formed by one molecule of βTGF binding to two 280 kilodalton receptor subunits.

1179 ACTIVATION OF C KINASE IN SWISS 3T3 CELLS, Patricia G. McCaffrey and Marsha Rich Rosner, Massachusetts Institute of Technology, Cambridge, MA 02139

In order to assess the state of activation of the Ca^{2+} and phospholipid stimulated protein kinase C in intact cells, we are characterizing the relationship between in vitro C kinase activity and a biological response, inhibition of epidermal growth factor (EGF) binding. Several activators of C kinase such as tumor promoters, diacylglycerol, and platelet-derived growth factor all cause increased overall phosphorylation of the EGF receptor and decreased EGF binding to its receptor in several cell types. In Swiss 3T3 cells, low concentrations of the tumor promoter phorbol dibutyrate (PDBu) maximally inhibit EGF binding even after 24 hours of continuous treatment. EGF binding is rapidly recovered upon removal of PDBu and cells are fully responsive to a subsequent dose of the tumor promoter. There is no change in the total cellular C kinase activity under these conditions. In contrast, when cells are treated with a higher dose of PDBu for 24 hours, an 85% decrease in the amount of total cellular C kinase is observed, and this loss is not rapidly recoverable. At the same time, PDBu still causes almost maximal inhibition of EGF binding. Thus, in Swiss 3T3 cells there appears to be a large excess of C kinase activity, such that only 15% of this activity is sufficient to obtain a nearly complete biological response. In addition, it appears that low level stimulation of C kinase can result in a long term biological response without causing inactivation of the enzyme. The role of these different states of C kinase activation in the response of cells to a variety of stimuli can now be determined.

1180 THE INFLUENCE OF RAS^H GENES ON METASTATIC POTENTIAL: Ruth J. Muschel, Doug Lowy and Lance A. Liotta, National Institutes of Health, National Cancer Institute, Laboratory of Pathology, Bethesda, MD, 20205

Cell lines transformed by various members of the ras^H family were tested for metastatic potential in the lung colonization assay. NIH-3T3 cells, transformed by either cloned Harvey Sarcoma virus (HSV) DNA or by the cloned T24 human oncogene, formed lung colonies after intravenous injection in nu/nu mice.

The normal cellular counterpart of the ras^H oncogenes transforms NIH-3T3 cells if an LTR is inserted upstream from the c-ras^H gene to boost expression of the normal P21, (Chang et al., Nature 307: 658, 1982). Such cell lines are tumorigenic, but do not form metastases. This confirms in this system that tumorigenicity and metastatic capacity are distinct phenotypic properties. Thorgeirsson et al., (Mol. and Cell. Biol., in press) have also found that foci of NIH-3T3 cells transfected with DNA from a leukemic cell line bearing N-ras are metastatic.

C127 cells transformed with v-ras^H or with recombinant constructs of HSV which lead to higher levels of P21 expression did not metastasize.

We speculate that we have identified two complementation groups which may be involved in the development of metastatic potential; one residing in some oncogenes and the other revealed by the difference in response of the C127 and the NIH-3T3 cells to transformation by v-ras^H.

1181 GENE AMPLIFICATION IN CHEMICALLY-INDUCED MOUSE SKIN TUMORS, John F. O'Connell, Andrew P. Butler and Thomas J. Slaga, University of Texas System Cancer Center, Science Park - Research Division, Smithville, Texas 78957.

Gene amplification has been proposed as a mechanism leading to increased expression of normal and/or altered gene products during carcinogenesis. We are using the two stage mouse skin carcinogenesis model to investigate possible gene amplification during a defined initiation - promotion protocol. The presence of amplified sequences in tumors derived from Sencar mice initiated with DMBA and promoted with 12-tetradecanoyl-13-phorbol acetate (TPA) was tested using Southern blot hybridization with middle repetitive and c-oncogene probes. Differential screening of DNA from untreated epidermis and carcinomas using genomic probes fractionated to a Cot value of 10-300 suggests the amplification of several discrete bands in some (but apparently not all) carcinomas. In addition, hybridization of both dot blots and Southern blots with c-DNA oncogene probes indicates possible amplification of the H-ras gene in some carcinomas. These results suggest that gene amplification does occur during two-stage chemical carcinogenesis. It is currently unknown if the observed amplification is a critiacl event in carcinogenesis. The possible relationship between gene amplification tumor progression is under investigation. In addition, a Sencar mouse keratinocyte cell line is being used to investigate possible mechanisms by which tumor promoters and carcinogens might mediate gene amplification. In this cell line, TPA appears to enhance amplification of the dihydrofolate reductase gene in cultures selected for methotrexate resistance, as has been previously reported for cultured fibroblasts. NIH support: CA 34890, CA 34962, CA 34521.

1182 ALTERED PATTERNS OF ONCOGENE EXPRESSION DURING TWO-STAGE SKIN CARCINOGENESIS. J.C. Pelling, G. Patskan, M. Junio, D.C. Hixson, R.S. Nairn and T.J. Slaga, The University of Texas System Cancer Center, Science Park, Research Div., Smithville, TX 78957

It has been demonstrated that carcinogenesis in a number of tissues and species can be divided into two stages, initiation and promotion. Furthermore, studies have shown that the stages involved in carcinogenesis are often accompanied by gene activation and/or altered patterns of gene expression. We have employed the two-stage skin carcinogenesis model in SENCAR mice to determine whether alterations in oncogene expression occur at various times during tumorigenesis in vivo. SENCAR mouse epidermis was screened at specific times during two-stage carcinogenesis using Northern blot hybridization analysis with a nick-translated [^{32}P]-cDNA probe for the Ha-ras oncogene. We observed increased levels of Ha-ras RNA in approximately 50% of papillomas and 2 of 3 carcinomas arising after initiation with dimethylbenz(a)anthracene (DMBA) and twice-weekly treatments with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). Only trace amounts of Ha-ras RNA were present in untreated SENCAR epidermis or in epidermis treated with DMBA or TPA alone. Activation of expression was also observed for the myc oncogene and the endogenous mouse murine leukemia virus env gene in a percentage of papillomas and carcinomas tested. Different phenotypes for the myc, Ha-ras and env genes were expressed in individual tumors, i.e., not all tumors expressing Ha-ras RNA were also expressing the myc or env gene RNA. These studies may enable us to establish whether activation or altered patterns of oncogene expression during two-stage carcinogenesis coincide with particular stages of tumor progression in vivo.

1183 INCREASED ONCOGENE EXPRESSION IN A MALIGNANT SCHWANNOMA, Molly R. Schwenn, Paul C. Billings, Ralph R. Weichselbaum and John B. Little, Dept. Cancer Biology, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115

Cytogenetic analysis of a malignant schwannoma in culture, obtained from an untreated patient with neurofibromatosis (NF), revealed double minutes, homogeneously staining regions, as well as marker chromosomes and heteroploidy. The present investigation was designed to determine whether there was increased expression of specific oncogenes in these cells. RNA was prepared from the schwannoma cell line and skin fibroblasts from the same patient, spotted onto nitrocellulose filters, and hybridized with N-myc, Ha-ras, Ki-ras, and B-lym probes. The schwannoma cells showed increased expression of N-myc. These cells are probably of neural crest origin like neuroblastomas, which show increased expression of N-myc and gene amplification in advanced cases. The schwannoma cells also exhibited increased expression of K-ras and B-lym compared with the skin fibroblasts. No difference in Ha-ras expression was observed. To our knowledge, this is one of the first reports to demonstrate increased B-lym expression in non-B-cell malignancies. We are currently looking at benign schwannomas and neurofibromas for increased expression of these oncogenes or a subset thereof, as well as looking at DNA for evidence of gene amplification or rearrangement.

1184 IDENTIFICATION OF A 5' FLANKING SEQUENCE OF THE C-HA-RAS-1 ONCOGENE WHICH IS A LOW-REPEAT IN MAMMALIAN DNA, Charles H. Scoggin, Cynthia S. Firnhaber, James H. Fisher, Eleanor Roosevelt Institute for Cancer Research and Webb-Waring Lung Institute, Denver, CO 80262

Particular oncogenes have been mapped to distinct locations on chromosomes using recombinant DNA probes derived from genomic DNA by using convenient restriction sites. Because of their method of derivation, oncogene segments contain flanking regions of associated DNA in addition to the transforming sequence of the oncogene. We have chosen to focus on areas outside of the transforming sequences, and to examine their representation in rodent and human DNA. We have found that a 0.85 kb fragment of DNA immediately 5' to the transforming sequence of the c-Ha-ras-1 oncogene yields multiple bands of molecular hybridization in human and hamster genomic DNA. Using somatic cell hybrids, we have been able to map specific bands to particular human chromosomes. This 0.85 kb fragment has been previously shown to contain enhancer sequences. The 0.85 kb fragment of the T24 oncogene corresponding to the probe utilized in this study has also been shown to contain v-myc related sequences. It may be speculated that this 0.85 kb fragment-containing enhancer sequence plays a role in the regulation of c-Ha-ras-1 oncogene expression and other genes with which it may be linked.

1185 TYPE β-TRANSFORMING GROWTH FACTOR/GROWTH INHIBITOR: A UNIQUE REGULATOR OF PROLIFERATION IN MESENCHYMAL AND EPITHELIAL CELLS, Gary D. Shipley and Harold L. Moses, Dept. Cell Biology, Mayo Clinic/Foundation, Rochester, MN 55905

Type β-transforming growth factor/growth inhibitor (TGFβ/GI) isolated from human platelets is a 25,000 dalton protein composed of two identical subunits with unique cell surface receptors on a variety of cell types. TGFβ/GI stimulates the formation of colonies by mouse AKR-2B cells and human diploid fibroblasts in soft agar in the absence of added EGF. TGFβ/GI also stimulates DNA synthesis in serum-free monolayer cultures of AKR-2B cells. Induction of DNA synthesis in monolayer cultures takes place after a prolonged prereplicative phase (∿24 hrs) when compared to other growth factors (EGF, FGF, PDGF) that induce DNA synthesis after a lag phase of approximately 13 hrs. The mitogenic activity of all of these growth factors is synergistic with insulin. TGFβ/GI inhibits the "early" peak (23 hrs) of EGF plus insulin stimulated DNA synthesis resulting in a peak of DNA synthesis at approximately the same time as the addition of TGFβ/GI alone (34-36 hrs). TGFβ/GI inhibits the growth of normal human keratinocytes and mammary epithelial cells in serum-free monolayer cultures and several human carcinoma cell lines in soft agar (in the presence of serum). The data suggest that TGF/GI may have an autocrine role in the development of mesenchymal tumors and stimulate the proliferation of stromal tissue in cancers of epithelial origin. (Supported by NCI grants CA 27217, CA 16816, and CA 09441.)

1186 Regulation of Human Metastatic Melanoma Cell Growth by Transforming Growth Factor β Nancy J. Sipes, Marvin D. Bregman, Frank L. Meyskens, Jr. AZ Cancer Center, Tucson Az 35724

The expression of the transformed phenotype is observed in vitro as anchorage-independent growth. Variability of cloning efficiency in soft agar among tumor samples may in part represent different capabilities of specific cells to respond to growth regulatory molecules provided by the plating medium. A human platelet sonicate was evaluated for effects on growth of human metastatic melanoma colony-forming cells in soft agar from cells in culture and from biopsies. These responses were quantitiated to determine changes in total cellular proliferation. The addition of platelet sonicate increased both the cloning efficiency (more colonies) and the proliferative capacity (larger colonies) of melanoma cells. Platelet-derived growth factor (PDGF), epidermal growth factor-like polypeptide (EGF), and transforming growth factor β (TGFβ) are three growth factor activities identified in platelets. The addition of PDGF and EGF alone and in combination did not increase soft agar colony formation. These studies indirectly suggest that TGF β is promoting the expression of the transformed phenotype. In order to evaluate this regulatory role of TGF β in human melanoma, we are purifying TGF β from platelets. Biological and biochemical studies of human melanoma cellular responses to TGF will be performed, and the results of these mechanistic studies will be presented.

1187 PURIFIED LOW AND HIGH MOLECULAR WEIGHT FORMS OF HUMAN EPIDERMAL GROWTH FACTOR CAUSE ANCHORAGE-INDEPENDENT GROWTH OF NRK CELLS, Kurt Stromberg, M.D.* and David Orth, M.D.**, *Laboratory of Viral Carcinogenesis, NIH, FCRF, Frederick, MD 21701, and **Division of Endocrinology, School of Medicine, Vanderbilt University, Nashville, TN 37232

Although epidermal growth factor (EGF) partially purified from murine salivary glands has been reported to elicit anchorage-independent growth (AIG) in normal rat kidney (NRK) cells, whether or not highly purified human EGF, in either its low (LMW) or high molecular weight (HMW) form can specifically cause AIG has not been examined. Consequently, LMW and HMW huEGF from human urine of normal donors was purified to homogeniety (D.N. Orth and C.D. Mount, unpublished data). Concentrations of 2.5 to 20 nanograms of LMW and HMW huEGF as measured by radioimmunoassay were tested for AIG of NRK cells (SA_6 clone, passage 12 to 14). Both forms of huEGF resulted in a plateau of AIG of NRK cells of colonies greater than 20 cells. Larger colonies of over 50 cells were obtained after addition of βTGF, kindly provided by Dr. Michael Sporn.

Rabbit antiserum raised against purified LMW huEGF was able to specifically inhibit AIG induced by both LMW and HMW huEGF in a dose specific manner in either the presence of absence of βTGF. A commercially available antiserum directed against a synthetic C-terminal portion of αTGF did not inhibit huEGF-induced AIG at the antiserum concentration range evaluated, nor did several other non-specific rabbit antisera.

In summary, both LMW and HMW huEGF in pure form are alone able to stimulate AIG in NRK cells which is specifically inhibited by huEGF antiserum.

1188 STIMULATION OF PROTEIN PHOSPHORYLATION IN PLASMA MEMBRANES OF HUMAN ACTIVATED LYMPHOCYTES BY INTERLEUKIN 2. Masaaki Taguchi and William L. Farrar, Laboratory of Molecular Immunoregulation, National Cancer Institute-FCRF, Frederick, MD. 21701

In a previous report IL-2 was shown to induce translocation of protein kinase C(PK-C) from cytosol to plasma membrane in murine IL-2 dependent T cells(Farrar and Anderson, Nature, in press). Among the membrane-associated proteins phosphorylated by PK-C was the IL-2 receptor identified by anti-Tac monoclonal antibody immunoprecipitation(Taguchi, et al. submitted). PK-C enzyme activation requires the presence of Ca^{2+} and phospholipids as co-factors, the data presented in this study identifies an additional phosphotransferase system that is distinct from calcium/phospholipid-dependent PK-C activation. IL-2 dependent lectin-activated human T lymphocytes were homogenized and plasma membranes isolated using sucrose discontinuous gradient ultracentrifugation(100,000xg, 1 hr.).Prepared membranes were preincubated at 4°C with or without homogenous recombinant human IL-2 for 10 min. The reaction mixture contained HEPES buffer pH 7.6, Mg^{2+}, Mn^{2+}, EGTA and was initiated by the addition of ^{32}P-ATP. Following incubation for 5 min. at 30°C the reaction was terminated with isoelectrofocusing sample buffer and urea. The samples were analyzed by O'Farrell's two-dimensional electrophoresis and phosphoproteins visualized by autoradiography. IL-2 induced phosphorylation of basic proteins (80 kd and 38 kd)and several membrane-associated acidic proteins(120 kd, 16 kd,and 13 kd).The 80 kd protein was phosphorylated at threonine residues. Several of the phosphoproteins had chemical characteristics similar to actin and synapsin. Since this protein kinase system is independent of PK-C, the evidence suggests that IL-2 may induce multiple kinase systems as a result of IL-2 receptor interation.

1189 **TUMOR PROMOTERS INHIBIT RADIATION-INDUCED LETHAL DNA FRAGMENTATION IN MOUSE EMBRYONIC FIBROBLASTS, L. David Tomei, Peter Kanter, and Charles E. Wenner Comprehensive Cancer Center, Ohio State University, Columbus, Ohio and Roswell Park Memorial Institute, Buffalo, NY 14263**

In view of our earlier studies which indicated that cytotoxic stress (such as acute serum deprivation) induced DNA fragmentation (Kanter et al BBRC 118 393, 1984), it was of interest to learn whether these agents also prevented radiation-induced DNA fragmentation (e.g. with high specific activity tritiated thymidine). Our data revealed that when the DNA fragmentation index was measured by formamide-gel fractionation, the % double stranded DNA was lowered by ^{3}H-dThd (80 curies/m mole- 1 μCi/ml). Damage appeared after 24 h which progressed to 96 h ranging from 6% at 24 h up to 51% at 96 h. Both TPA (10^{-7}M) and DHTB (10^{-8}M) reduced fragmentation by as much as 90%. In order to determine whether fragmentation was dependent on protein synthesis as expected in programmed cell suicide, the effects of cycloheximide were examined. Results indicated that 10^{-5}M cycloheximide also inhibited to the same degree observed with tumor promoters. High specific activity ^{3}H thymidine incorporation into cellular DNA leads to extensive DNA fragmentation which is apparently dependent on protein synthesis and is inhibitable by tumor promoters. The above findings are consistent with the concepts that radiation lethality is a consequence of induction of programmed cell suicide (apoptosis), and tumor promoters may act by interfering with this physiological process. (with technical help of L. Bartels) Supported in part by CA-13784.

1190 PURIFICATION AND PROPERTIES OF THE HUMAN DNA METHYLTRANSFERASE. Keith E. Zucker, Arthur D. Riggs, and Steven S. Smith, Beckman Res. Institute of the City of Hope, Duarte, CA 91010.

Abnormal DNA methylation patterns are often found in transformed cells and cell lines. In general, these patterns are hypomethylated relative to normal tissue, and may be involved in the altered transcriptional patterns often associated with transformed cells. This suggests that at least a transient interruption of the normal methylation process has occurred during their generation. A key point at which this process might be interrupted is the DNA methyltransferase that appears to be responsible for somatic inheritance of methylation patterns in DNA. We have developed a facile procedure for the purification of this enzyme from human placenta. It has been purified approximately 38,000-fold from a crude whole-cell extract by means of ion exchange, affinity, and hydrophobic interaction chromatography; followed by prepartive glycerol gradient centrifugation. It appears to be homogeneous, is composed of a single polypeptide chain of 126 kd, and prefers hemimethylated DNA over native DNA by about twelvefold. The availability of highly purified human DNA methyltransferase in large amounts should facilitate many studies on the structure, function, and expression of these activities in both normal and transformed cells.

Liver Cancer

1191 HBV DNA REPLICATION STATE AND LIVER DISEASE ACTIVITY DURING PERSISTENT INFECTION AND HEPATIC ONCOGENESIS, David A. Shafritz, Robert D. Burk, Michael C. Kew and Stephanos J. Hadziyannis, Liver Research Center, Albert Einstein College of Medicine, Bronx, NY, 10461, Johannesburg, South Africa and Athens, Greece.
Epidemiologic and molecular studies have shown a strong correlation between persistence of hepatitis B virus (HBV) infection and development of hepatocellular carcinoma (HCC). Most tumors from HBV carriers show integrated HBV DNA molecules in discrete bands on Southern blot analysis, characteristic of monoclonality or oligochonality of tumor cell populations. Integration of HBV DNA diffusely throughout the liver genome or as discrete bands has also been reported in HBV carriers without HCC. However, events leading to HBV DNA integration and subsequent hepatic oncogenesis have not been well defined. To understand this process, we have compared the replication state of HBV in liver tissue of long-term carriers to the level of liver disease activity, the integration of HBV genomes and the development of HCC. Histologic, cellular and molecular studies indicate that persistent HBV infection falls into three categories, permissive, non-permissive or mixed, in which replicating and nonreplicating states are found in different cells or regions of the same liver. Features of replicating or permissive infection include active inflammatory liver disease (chronic persistent or chronic active hepatitis), HBsAg and HBcAg production by individual hepatocytes (distributed randomly throughout the liver parenchyma) and both free virions and lower molecular weight forms characteristic of replicating HBV DNA. Features of non-replicating or non-permissive infection include absence of active inflammatory liver disease, continued HBsAg but not HBcAg production, and presence of integrated HBV DNA. HBsAg production is often present in groups or clusters of hepatocytes having the nodular appearance of a focal clonal growth. In several of the latter cases, we have noted integration of HBV DNA into the same host site in many cells (unique bands on Southern blot analysis) again suggesting a monoclonal origin of cells which have already begun to divide. Therefore, in non-permissive infection, the presence of integrated HBV DNA in unique bands may represent a significant step in the transformation process. Carriers from Greece with HCC often show continued HBV replication, whereas carriers from South Africa with HCC show suppressed HBV replication. In addition, South African patients develop HCC at a much earlier age than Greek or Oriental patients, often have no evidence of underlying liver disease and consistently show multiple discrete, incomplete or rearranged HBV genomes integrated into tumor cell DNA. In three South African patients, we have found high levels of HBV genomes in liver or tumor cells but no release of virions into the serum. These findings are consistent with defective HBV replication or a block in viral assembly and suggest that such defectiveness might be associated with an increased propensity for HBV DNA integration and subsequent development of hepatic malignancy.

1192 MOLECULAR STUDIES OF HEPATITIS B VIRUS, G.H. Yoakum, C.C. Harris, B.E. Korba, D.C. Boumpas, D.L. Mann, Sun Tsung-tang*, Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD 20205, and *Cancer Institute, CMIS, Beijing, Peoples Republic of China
Studies of Hepatitis B virus at the molecular level have yielded important information about the integration of viral DNA into the cell genome, the persistence and tissue distribution of HBV in patients, and the expression of HBV genes including the mode of regulation and biological effects of HBV gene products. The HBV core antigen gene (HBc) encodes a 184 amino acid polypeptide (HBcAg) that is associated with the more severe consequences of HBV infection including: chronic active hepatitis (CAH), a highly infectious status, cirrhosis of the liver, and virus DNA replication. Transfection studies to elucidate the mechanism of regulation of HBcAg expression and the biological consequences of HBc gene expression were initiated by the construction of a recombinant human cell line (GTC2). GTC2 is a mucoepidermoid carcinoma cell that contains one complete copy of the HBc and gpt genes. The GTC2 cell line was used to determine that the expression of the HBcAg *in vitro* requires growth in a complex medium for the recombinant GTC2, and an hepatocellular carcinoma cell line PLC/PRF/5 (Alexander). HBcAg expression is also regulated by the methylation status of a Hpa II site located 280 base pairs upstream from the HBc structural gene ($\underline{Hpa}\ II^{-280}$). The demethylation of $\underline{Hpa}\ II^{-280}$ in the cell population is completed approximately 6 cell divisions after 5^1-azacytidine treatment, and leads to high level expression of the HBc gene when human cells carrying the hypomethylated gene are grown in LHC-4 medium. Expression of the HBc gene in human epithelial cells causes cytopathological effects that reach a cytolytic stage when the HBc gene is induced before expression, or when multiple copies of the gene available for expression. The role of HBc gene expression in HBV infection may include a direct role in the cytolytic destruction of infected cells. Therefore, HBV may act as a promoter during liver carcinogenesis, that could interact with other viruses and/or chemical agents that may initiate hepatocellular carcinoma. The recent observation that mononuclear blood cells and lymphnode tissue from CAH, lymphadenopathy, and AIDS patients frequently contain HBV DNA, and RNA, suggests that the immune system may be involved in the pathologic effects that occur during HBV infection: i) infected lymphocytes may be a reservoir for transmission of the disease and re-expression of the virus after extended latency periods between clinically observable infections, ii) the cytolytic effects of HBc gene expression

suggests the potential alteration of the host immunological response by direct cytolysis of immunologically active subsets of mononuclear blood cells. Current studies on the effect of alpha interferon on HBV gene expression indicate that interferon treatment of HBV-carrying human cells in tissue culture decreases the expression of the HBsAg gene at the transcriptional level, and stimulates the expression of HBc gene transcripts.

Growth Factors and Transforming Growth Factors

1193 RETROVIRUSES CODING FOR POLYOMA VIRUS EARLY REGION TRANSFORMATION ANTIGENS - T ANTIGEN SPECIFIC CHANGES IN GROWTH FACTOR REQUIREMENTS, Van Cherington, Bill Morgan, and Thomas M. Roberts, Dana-Farber Cancer Institute, Boston, MA. 02115

The early region of polyoma virus, a DNA tumor virus, codes for three proteins, called large T(100K daltons), middle T (56K daltons), and small T (21K daltons) antigen, which together contain all of the information needed to transform primary cells in vitro and form tumors in vivo. Middle T alone is sufficient for transformation of immortalized fibroblasts, although small T may potentiate this activity. Because retroviruses are extremely efficient vectors for transducing genetic material into a wide range of recipient cells, we have constructed recombinant murine retroviruses which code for individual polyoma T antigens in order to study the effect of the expression of these proteins individually in a wide variety of cell types. We are utilizing a Moloney murine leukemia virus-derived vector (Cepko et al., 1984, Cell 37: 1053) which contains the coding region for neomycin resistance (neo^r). Retroviruses coding for small T antigen have been constructed and their characterization is in progress. Large T antigen retroviruses have no clear effect on the growth regulation of NIH3T3 cells. Middle T antigen retroviruses are sufficient to transform fibroblast lines to form foci in monolayer cultures. Focus formation and neo^r were transferred with equal efficiency by the middle T virus. Middle T retrovirus transformed C3H10T1/2 mouse cells, a C57 black mouse cell line and Fisher rat 3T3 cells, as efficiently as NIH3T3's. Middle T, but not large T, retrovirus infection eliminated the growth requirement for epidermal growth factor in a defined medium.

1194 CHARACTERIZATION OF THE MELANOMA MONOLAYER MITOGEN, MGSA, USING MONOCLONAL ANTIBODIES. Ann Richmond, David H. Lawson, Juanester Lamb, Chris Engel, Robert Roy. Department of Medicine, Emory University and V.A. Medical Center, Atlanta, Ga. 30322

The growth of the human melanoma cell line Hs0294 in serum free culture medium is associated with production of an autostimulatory monolayer mitogen. This melanoma growth stimulatory activity (MGSA) is stable to acid and heat. Trypsinization as well as reduction with dithiothreitol result in loss of the mitogenic activity. MGSA can be partially purified from acetic acid extracts of Hs0294 conditioned medium (CM) by Bio-Gel P-30 chromatography followed by RP-HPLC on a μ Bondapak C_{18} column. A monoclonal antibody (FB2AH7) which recognizes an antigen in the RP-HPLC purified MGSA preparation has been developed. This antibody inhibits the growth of Hs0294 cells in serum free culture medium, has no effect on the growth of NRK cells in serum free medium, but inhibits the mitogenic response of NRK cells to Hs0294 CM. Mitogenic activity can be recovered from an FB2AH7 immunoaffinity column after elution with 0.5 M sodium acetate, pH 4.5. Immunoprecipitates of ^{35}S-methionine labeled Hs0294 cell extracts subjected to SDS-PAGE under reducing conditions were visualized by fluorography. The data obtained from these experiments suggest that the FB2AH7 antigen is synthesized as a >20 Kd precursor which then undergoes proteolytic processing. The relatively broad elution profile of MGSA after Bio-Gel P-30 chromatography along with the elution of one major and several minor active peaks after RP-HPLC suggest that these processed forms of the antigen retain mitogenic activity. Supported by NCI Grant Number IR23 Ca 34590 and Veterans Administration Merit Award #RDIS7444-01.

Liver and Lung Cancer; Detection of Oncogenes in Viruses in Human Tissues and Cells

1195 TRANSPLACENTAL CARCINOGENESIS AND MULTIHIT THEORIES OF TUMOR DEVELOPMENT, D.G. Branstetter, The Upjohn Co., Kalamazoo, MI., P. Conran, P.J. Goldblatt, Medical College of Ohio, Toledo, OH.

A widely accepted concept in the area of carcinogenesis is that multiple insults or "hits" must be received by an initially normal cell in order for it to be transformed over time into a clinically detectable and malignant neoplasm. Epidemiological studies of cancer incidence in man during aging and experimental studies in animals have also prompted numerous mathematical models of carcinogenesis which indicate that multiple discrete events must occur in the course of malignant tumor development. In transplacental carcinogenesis studies performed in the mouse, we have observed that liver and lung tumors induced early in organogenesis are much larger than those induced late in gestation or in adult mice. The proportional decrease in tumor size observed when they are induced at later stages of development roughly parallels the decrease in fetal growth rate during this same period and the large tumors induced early in gestation are composed of aggregations of smaller "adult" sized tumors. This suggests that many descendants of a single transformed fetal cell retain the capacity to become tumors in later life. If this is true, either the probability of additional "hits" occurring after the initial carcinogen related hit is fairly high or only a single hit is required for mouse liver and lung tumor development.

1196 STRUCTURE AND TRANSCRIPTION OF THE C-RAF-1 ONCOGENE LOCUS, John L. Cleveland, Thomas I. Bonner, Mindy D. Goldsborough and Ulf R. Rapp, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701

We have isolated and characterized molecular clones containing the human and murine homolog (c-raf) of the 3611 MSV and MH2 oncogenes (v-raf/mil). Two human genes homologous to raf/ mil have been cloned and sequenced. One, c-raf-2, is a processed pseudogene; the other, c-raf-1, contains nine exons homologous to both raf and mil and two additional 5' exons homologous to mil. Hybridization mapping using a human c-raf cDNA (Bonner et al., in preparation) probe revealed that sequences homologous to c-raf cDNA are contained in at least 15 exons and span more than 44 kilobase pairs. A 3' portion of human c-raf-1 containing six of the seven amino acid differences relative to v-raf can substitute for the 3' portion of v-raf in a transformation assay. The transforming ability of c-raf has also been demonstrated using a recombinant containing the murine c-raf hooked onto the LTR of 3611 MSV. Sequence homologies between human and murine c-raf and Moloney leukemia virus at both ends of v-raf indicate that the viral gene was aquired by homologous recombination, probably at the DNA level. Using Northern hybridization analyses with exon and intron-specific c-raf-1 probes, we have obtained evidence for three alternatively spliced poly(A)$^+$ RNAs and it appears that expression of one message, containing the most 3' portion of c-raf, is associated with transformation. This hypothesis is currently being tested by analyses of cDNA clones.

1197 FAMILIAL CLUSTERING OF MALIGNANCIES: DISTRIBUTION OF ONCOGENE POLYMORPHISMS IN NORMAL AND HIGH-RISK POPULATIONS. J.C. Cohen, W. Ooi, J. Bickers, G. Berenson, P. Bloemers, and H. Rothschild, Louisiana State University, New Orleans, LA and University of Nijmegen, Nijmegen, The Netherlands

In a retrospective study using deceased probands with lung cancer, a familial clustering of malignancies was documented. In these families a significant correlation beteen relationship to the proband and the occurrence of lung cancer was demonstrated. This relationship was independent of smoking history. Of interest was the observation that several other cancers clustered in these families. These data suggest that a common genetic determinant is responsible for the increased frequency of malignancy. The promoting effect of carcinogens present in cigarette smoke would enhance the prospect for lung cancer in these families.

As an initial step in determining the genetic and biochemical basis for this clustering we have examined genetic polymorphisms in two oncogenes, Ha-ras and fms, in a retrospective study using living lung cancer probands. In a cross-sectional sample of 500 normal individuals we have determined the distribution of restriction fragment length polymorphisms for these two genes. Gene frequency as well as sex and race distributions were determined. The frequency was compared to that observed in DNA samples obtained from several high risk families to determine if a specific oncogene polymorphism was significantly associated with familial clustering.

1198 THE EFFECT OF SUBCHRONIC EXPOSURE OF MICE TO 1,1-DICHLORO-2,2-BIS(4'-CHLOROPHENYL)ETHENE (DDE): ALTERATION IN THE ENZYMES INVOLVED IN THE REGULATION OF REACTIVE OXYGEN SPECIES, Barry Gold and Galen Brunk, Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, NE 68105

The pesticide 1,1,1-trichloro-2,2-bis(4'-chlorophenyl)ethane (DDT) is a liver tumorigen in the mouse. It is only marginally active in the rat liver and inactive in the hamster. A major lipophilic metabolite of DDT is 1,1-dichloro-2,2-bis(4'-chlorophenyl)ethene and it is a hepatocarcinogen in both the mouse and hamster. Since it has been demonstrated that a major difference between the mouse and hamster is that the former is far more efficient than the latter in metabolizing DDT to DDE, it is reasonable to suggest that DDE may be the proximate form of DDT. We have also demonstrated that neither DDT nor DDE show any indication of being acutely genotoxic. Because of the number of reports indicating that the generation of reactive oxygen species may be involved in certain stages of tumorigenesis, the effect of DDE on the key enzymes involved in the regulation of reactive oxygen species was studied after subchronic exposure to the compound. DDE was administered in food at a level of 250 ppm (5 nmole/mouse/day) for a period of 4 months. Untreated animals were similarly maintained. Animal weights and food consumption did not differ between treated and untreated groups. DDE had no effect on the level of catalase, or total and selenium-dependent glutathione peroxidases. However, there was a 25% decrease in the level of CuZn superoxide dismutase in DDE-treated animals. The biological significance of this finding is under investigation and will be discussed.

1199 THE TUMORIGENICITY AND TRANSCRIPTIONAL MODULATION OF C-MYC AND N-RAS ONCOGENES IN A HUMAN HEPATOMA CELL LINE, Brian Huber, Kerry Dearfield*, Jerry Williams*, Carole Heilman and Snorri Thorgeirsson, Laboratory of Experimental Carcinogenesis, NCI, Bethesda, MD 20205 and The George Washington University Medical Center*, Washington, DC 20037

We have examined the tumorigenicity and oncogene expression in a human hepatoma cell line, HepG2. HepG2 cells and a single cell clonal HepG2 line, HLD2-6, were equally tumorigenic when injected s.c. into athymic nude mice. Cyclophosphamide (CY) pretreatment of both cell lines had no effect on tumor incidence or latency despite inducing sister chromatid exchange (SCE). Tumors were nonencapsulated, highly invasive adenocarcinomas and were positive for γ-glutamyltranspeptidase activity and bile formation. Plasma from tumor-bearing mice were positive for human α-fetoprotein and negative for Hepatitis B Virus (HBV) surface antigen. Reestablished cell lines from HLD2-6 derived tumors had cell cycle times comparable to HLD2-6 cells but decreased SCE induction after CY treatment. C-myc transcription, when compared to a normal human liver sample, was increased > 10 fold in all HLD2-6 cell lines and HLD2-6 derived tumors. This increase in c-myc expression could not be explained by gene amplification or site specific HBV integration. N-ras transcription in cultured HLD2-6 cells was comparable to a normal human liver sample, but there was a selective 3 to 5 fold increase of the 5.5 kb N-ras transcript in HLD2-6 tumors grown in nude mice. The 5.5 kb transcript decreased upon reestablishing the tumor cells in tissue culture to a level comparable to that found in the normal human liver sample, suggesting the existence of modulating factors in the host animal.

1200 CHRYOTILE ASBESTOS INDUCTION OF DNA SYNTHESIS PERTURBATION AND OF 68K PROTEIN IN CULTURED HUMAN LUNG CELLS, R.C. Johnson, Dept. BCIM, Medical University of S.C. Charleston, S.C. 29425

Because the evidence for chrysotile asbestos induced DNA damage in cultured human lung cells is inconclusive, the concept of asbestos as a promotor or participant in some epigenetic role has been encouraged. However, recent studies (Hesterberg and Barret, 1984, Cancer Research 44:2170) suggest that asbestos acts by itself to transform cultured mammalian cells, similarly to classic mutagen/carcinogens. In this study cultured human lung cells were exposed to concentrations of asbestos that demonstrated no overt effect on the rate of cell proliferation, but appeared to perturb normal patterns of DNA synthesis assembly as seen on alkaline sucrose gradients. In addition, this treatment induced a 68K protein that may be the same as the 68K basic protein particular to induction by classic mutagens in cultured human cells. A DNA repair deficient strain of human fibroblasts has been observed for its sensitivity to asbestos. The above results raise the possibility that asbestos may share some pathway with the proposed inducible repair and/or mutation pathway of classic agents that initiate events by DNA synthesis perturbation.

1201 QUANTITATIVE RELATIONSHIP BETWEEN INITIATION OF HEPATOCARCINOGENESIS AND INDUCTION OF ALTERED CELL ISLANDS. W.K.Kaufmann, S.A.MacKenzie, R.J.Rahija and D.G.Kaufman, Department of Pathology, University of North Carolina, Chapel Hill, NC 27514.

Our recent studies have quantified the induction of islands of histochemically-altered hepatocytes and the initiation of hepatocytic neoplasms. For up to 20 weeks after treating regenerating livers once with methyl(acetoxymethyl)nitrosamine (DMN-Ac), hepatocytic neoplasms were not seen. Thereafter in rats fed the liver tumor promoter, phenobarbital, neoplasms emerged continuously so that by 60 weeks after initiation livers held an average of 5.5 neoplasms. Islands of cellular alteration, identified by their abnormal retention of glycogen on fasting, also appeared to emerge continuously between 20 and 60 weeks. By 60 weeks promoted livers contained about 10,000 islands. Islands and neoplasms maintained a constant numerical relationship over time with 1600 islands emerging for every neoplasm that emerged. This ratio of islands to neoplasms appeared to vary according to the type of carcinogen used to initiate hepatocarcinogenesis and whether promotion was included. In livers initiated with DMN-Ac but not promoted with phenobarbital, the ratio of islands to neoplasms was about 12,000 to 1. In livers initiated with benzo[a]pyrene diolepoxide I and then promoted with phenobarbital the ratio of islands to neoplasms was 8000 to 1; in livers initiated with gamma rays and promoted this ratio was greater than 16,000 to 1. These results reveal a highly variable relationship between the numbers of putative "initiated" hepatocytes as represented by the island population and the numbers of initiated hepatocytes that can be promoted to form neoplasms. Supported by NIH grant #CA32238.

1202 CHROMOSOMAL SITE OF HBV INTEGRATION IN A HUMAN HEPATOCELLULAR CARCINOMA-DERIVED CELL LINE, B.B. Knowles, D. Simon, D.B. Searls, Y. Cao and K. Sun, The Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104

The single site of integration of hepatitis B virus in the human hepatocellular carcinoma cell line, Hep 3B 2-1/7, was found to segregate with human chromosome 12 in somatic cell hybrids. Analysis of metaphase spreads of Hep 3B 2-1/7 following in situ hybridization with pHBV revealed integration at 12q13-14, a location that coincides with the fragile site (fra 12 q13). The possible significance of this location to the development of hepatocellular carcinomas is discussed.

1203 EFFECTS OF TREATING MICE WITH BUTYLATED HYDROXYTOLUENE, A MODULATOR OF LUNG TUMOR FORMATION, ON THE PROTEIN KINASE C-CATALYZED PHOSPHORYLATION OF AN ENDOGENOUS 36K LUNG PROTEIN. Alvin M. Malkinson, Deborah S. Beer, Albert J. Sadler, and Donna S. Coffman. School of Pharmacy, University of Colorado, Boulder, CO 80309 and Chemistry Department, Colorado College, Colorado Springs, CO 80903.

Treatment of mice with the common food additive, butylated hydroxytoluene (BHT), modulates the carcinogenic effect of urethan on lung tumor production. This can take the form of prophylaxis, co-carcinogenesis, or promotion, depending on the experimental protocol. Protein kinase C (Pk-C) may mediate such modulations on tumor growth, as suggested by observations that Pk-C is the receptor for phorbol ester promoting agents and by the phosphorylation of epidermal growth factor receptor by Pk-C. We have found that the endogenous phosphorylation of a 36K protein (p36) in cytosolic fractions of mouse lung requires Ca^{++} and phosphatidylserine (PS). Phosphate was transferred to seryl and threonyl residues of p36 but not to tyrosyl residues. These are the characteristics of Pk-C catalysis. Short-term treatment of mice with single ip injections of either urethan or BHT causes proliferative changes in the lung. Urethan administration had no apparent effect on p36 phosphorylation. Treatment with BHT, however, reduced p36 phosphorylation while simultaneously increasing the Ca^{++}- and PS-independent phosphorylation of 33K and 30K proteins. These changes were maximal at the time interval following BHT injection corresponding to maximal hyperplasia. The mechanisms of these changes are under study. (Supported by USPHS grants ES02370, CA33497 and by RCDA CA 00939 to A.M.M.)

1204 SMALL CELL CARCINOMA OF THE LUNG: ISOLATION OF NORMAL AND ABNORMAL CHROMOSOME 3 IN SOMATIC CELL HYBRIDS AND IDENTIFICATION OF A DELETED GENE. Y.E. Miller, C.H. Scoggin and D. Palmer, Eleanor Roosevelt Institute for Cancer Research and Veteran's Administration Medical Center, University of Colorado Health Sciences Center, Denver, CO.

Cytogenetic studies of small cell carcinoma of the lung have revealed a distinct and reproducible abnormality: tumor cells contain an abnormal chromosome 3p del (14-23) in addition to one or more normal copies of chromosome 3. Somatic cell hybrids have been constructed using the Chinese hamster ovary auxotrophic mutant $U^{-}C$ and the NCI H69 small cell carcinoma line. Two hybrids, Y-195-1 and Y-195-4, were obtained which contain the deleted chromosome 3 and a normal human chromosome 3, respectively, in addition to other human chromosomes. In addition, the 314-2 hybrid, containing only a chromosome 3 from a normal human lymphocyte, has been constructed. All three hybrids were analysed for chromosome 3 markers with the following results:

HYBRID	CHROMOSOME 3	TRANSFERRIN RECEPTOR	1C5.3A.1 Cell SURFACE ANTIGEN	ACYLASE 1
314-2	Intact	+	+	+
Y195-1	del p (14-23)	+	+	-
Y195-4	Intact	+	+	+

The findings are consistent with previously reported regional localization data and confirm the reported deletion.

1205 PHOSPHOTIDYLINOSITOL KINASE ACTIVATION DURING CHEMICAL HEPATOCARCINOGENESIS. Jack W. Olson, College of Pharmacy, University of Kentucky, Lexington, Ky. 40536. Phosphotidylinositol 4-phosphate (PIP) and phosphotidylinositol 4,5-biphosphate (PIP_2) play key roles in signal transduction for several hormones and growth factors that activate cell proliferation. Tyrosine protein kinases associated with the insulin receptor and src and ros oncogene products are also phosphotidylinositol kinases (PIK). Activation of PIK could increase PIP and PIP_2, thus enhancing proliferation of responsive cells. Because proliferation is an essential component of carcinogenesis and liver tyrosine kinase is activated during hepatocarcinogenesis, liver PIK activity was determined at several stages of the Solt and Farber chemical hepatocarcinogenesis model. Rats were given diethylnitrosamine (DEN) in one dose (200 mg/kg), followed by 2 weeks of dietary 0.02% 2-acetylaminofluorene starting at day 14 after DEN and a partial hepatectomy on day 21. This complete regimen produces a synchronized population of hyperplastic nodules at day 32 and hepatocarcinomas at about 1 year. Rats were killed, liver or hepatocarcinoma homogenized, centrifuged at 1000 g for 10 min, the supernatant spun at 30,000 g for 30 min and the pellet assayed for PIK activity using exogenous PI as substrate. At day 32, liver PIK activity was 1.5 fold greater ($p<.05$) in rats that received the complete regimen versus sham or partial regimens. At 15 months, hepatocarcinoma PIK activity was 2.2 fold greater than the surrounding liver (96 vs. 212 pmol PIP/min/mg protein $p<.05$). This data demonstrates that increased liver PIK activity is associated with the very early stages of chemical hepatocarcinogenesis as well as hepatocarcinomas. Supported in part by PHS grant number (CA31099, awarded by the National Cancer Institute, DHHS.

1206 *Ras GENE ACTIVATION IN SPONTANEOUS MOUSE HEPATOCELLULAR TUMORS*, Steven H. Reynolds*, Jill Stowers*, Marshall Anderson*, Robert R. Maronpot+ and Stuart A. Aaronson¶, *National Institute of Environmental Health Sciences and +National Toxicology Program, Research Triangle Park, NC 27709; ¶National Cancer Institute, Bethesda, MD 20205

The development of cancer appears to involve a stepwise process with a general tendency to evolve cells with a more malignant phenotype. Although the process of malignant transformation is poorly understood, experiments have shown that the malignant phenotype can be transferred to NIH/3T3 fibroblasts by transfection of DNA from various tumors and tumor cell lines. We report here that DNAs from both spontaneous mouse hepatocellular adenomas and spontaneous mouse hepatocellular carcinomas are capable of inducing morphological transformation of NIH/3T3 fibroblasts. Approximately 30% (3/10) of the hepatocellular adenomas and 85% (11/13) of the hepatocellular carcinomas scored as positive by DNA transfection. The transforming properties of the mouse tumor DNA are the result of an activated cellular *ras* gene. Studies to be presented define the *ras* gene(s) implicated and the mechanisms involved. Our findings extend previous evidence that *ras* genes are commonly activated in spontaneous tumors and that activation can occur at a relatively early stage in the neoplastic process.

1207 DELETION IN CHROMOSOME 11p ASSOCIATED WITH HEPATITIS B VIRUS INFECTION. C.E. Rogler*, T.B. Shows+ and A. Henderson++. *Albert Einstein College of Medicine, Bronx, NY 10461, + Roswell Park Memorial Institute, Buffalo, NY 14263, ++ Hunter College, The City University of New York, NY 10021.
Chronic infection with hepatitis B virus (HBV) incurs a very high lifetime risk of hepatocellular carcinoma. HBV integrations are observed in the genomic DNA prepared from primary hepatomas of chronically infected humans. Molecular cloning of several of these integrations has been performed and analysis of the DNA has shown that integration can occur at variable sites in both the cellular and viral DNA. Analysis of cellular sequences immediately flanking HBV integrations has not revealed the presence of any known oncogenes or a common cellular integration site in independent tumors. Unique cellular sequences to the left and right of a cloned HBV integration were used as probes to construct a restriction endonuclease map of the cellular DNA sequences at the HBV integration site in the original tumor and the normal homologous cellular allele. This analysis revealed a deletion of at least 13.5 kb of cellular sequences at the HBV integration site. Southern blot analysis using a mouse-human somatic cell hybrid panel localized the deletion to chromosome 11p. In situ cytological hybridization independently localized the HBV integration to chromosome 11p13-11p14. Wilms tumor has also been associated with deletions at chromosome 11p13. Several genes involved in growth control are also located on chromosome 11p including insulin, insulin-like growth factor II, parathyroid hormone and c-Ha-rasI. Further work is in progress to investigate the possible role of chromosome 11p in hepatic oncogenesis.

1208 PROLACTIN MAY BE AN ENDOGENOUS TUMOR PROMOTOR. A.R. Buckley, M.S., C.W. Putnam, M.D., D.H. RUSSELL, Ph.D. Prolactin (PRL) rapidly induces ornithine decarboxylase (ODC) and plasminogen activator activities (PA), known enzymatic markers of a trophic response elicited by carcinogens, tumor promoters, and other stimuli. This suggested that PRL might function as a tumor promoter. To test this theory, Wistar Lewis rats were initiated with diethylnitrosamine (1nmol/kg, DEN) by intragastric gavage or vehicle. One week later, and thrice weekly for 6 weeks, ovine PRL (11mg/kg) or vehicle was administered (i.p.). Organ/body weight ratios, and hepatic γ-glutamyltranspeptidase (GGTase) activity and GGTase-positive foci were measured.

Results:	GP	DEN	PRL	n	Liver/Body Wt	GGTase IV/mg prot
	I	–	–	9	3.08±0.04	0.30±0.03
†p<0.02	II	–	+	8	3.25±0.05*	0.27±0.03
*p<0.01	III	+	–	5	3.16±0.08	0.47±0.06†
**p<0.001	IV	+	+	7	3.41±0.06**	0.58±0.09**

Additional animals received PRL at 12h intervals for 48 h. Hepatic DNA synthesis was measured by ^{3}H-thymidine incorporation; DNA synthesis was 182% ($p<0.05$) of controls. Conclusions: PRL administration results in hepatic hypertrophy and hyperplasia. Initiation plus PRL elevated hepatic GGTase activity and resulted in more detectable GGTase-positive foci. Together with the demonstrated effects of PRL to elevate ODC and PA, these data strongly implicate PRL as a tumor promoter. Thus, hyperprolactinemia may be predisposing to the development of neoplastic lesions by chemically initiated cells.

1209 RAS ONCOGENE IS DIFFERENTIALLY EXPRESSED DURING PROGRESSION OF DMBA-INDUCED CARCINOGENESIS, S.A. Schwartz, C.F. Shuler, R. Mostofi & M. Stein, U. of Chicago, IL. 60637
The application of DMBA to the hamster cheek pouch has long been employed to study the induction and progression of epidermal carcinoma. Because of the accessibility of this organ to periodic, noninvasive examination, workers have described in histopathological terms, the differential progression of the normal mucosa through increasing degrees of dysplasia, to invasive squamous cell carcinoma. It was of interest to determine whether oncogenes were expressed during this transition, and whether oncogene products were co-ordinately synthesized with other tissue-specific markers indicative of a terminally-differentiated state. Hamsters were given multiple intra-oral painting of DMBA, and tissues were periodically biopsied to determine: the histopathologic status of the oncogenic process; the extent of oncogene expression; and the electrophoretic composition of the tissue-specific cytokeratin intermediate filaments. Normal cheek pouch mucosa synthesized a characteristic family of keratins as determined by SDS-PAGE, without detectable levels of Ha-ras transcripts as determined by in-situ hybridization with a radiolabeled Ha-ras DNA probe. Subsequent hyperkeratotic areas revealed varying levels of epithelial hysplasia; PAGE disclosed the occasional loss of a higher MW keratin with the appearance of a new, lower MW species. Elevated expression of the Ha-ras oncogene was primarily limited to dysplastic epithelial cells. Invasive squamous cell carcinomas consistently demonstrated altered cytokeratin profiles in conjunction with markedly enhanced expression of Ha-ras. Our data suggest that p21 biosynthesis is co-ordinated with ongoing tumor progression and altered cell-specific expression

1210

LOCALISATION OF THE ONCOGENE C-ERBA1 IMMEDIATELY PROXIMAL TO THE APL TRANSLOCATION BREAKPOINT ON CHROMOSOME 17. D.Sheer, D.M.Sheppard,M.M.Le Beau, J.D.Rowley, C. San Roman, and E.Solomon, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX,England

The 15q+/17q- chromosome translocation associated with Acute Promyelocytic Leukaemia (APL) is of particular interest as it has not been seen in any other type of malignancy and may occur in virtually every case of APL. We have previously localised the translocation breakpoint to 15q22 and 17q12-21 by analysis of interspecific somatic cell hybrids containing translocation chromosomes (Sheer et al, Proc. Nat. Acad. Sci.USA (1983)80, 5007), and have shown that the oncogene c-erbA1 maps to 17p11-q12-21 (Spurr et al, EMBO J. (1984) 3, 159). We have now localised this oncogene by in situ hybridisation of a c-erbA1 probe on metaphase chromosomes from a woman with a constitutional translocation, t(15;17), with the breakpoint on 17 at 17q11. In contrast to APL where c-erbA1 remains on the 17q- chromosome, the oncongene is translocated to the 15q+ chromosome, and therefore maps to the region immediately above the APL breakpoint on chromosome 17. Experiments are in progress to determine whether this oncogene is rearranged in APL.

1211

CHROMOSOMAL ABERRATION AND LACK OF EGF RECEPTOR EXPRESSION IN SMALL CELL CANCER OF THE LUNG, N. Shimizu*#, S. Gamou*, H. Harigai*, S. Hirohashi** and Y. Shimosato**, *Dept. Mol. Biol., Keio Univ. School of Med., Tokyo;#Dept. Mol. Cell. Biol., Univ. of Arizona, Tucson;**Dept. Pathology, Natl. Cancer Ctr.,Res. Inst., Tokyo.
Previously, Whang-Peng et al. reported that all the cell lines developed from small cell cancer of the lung (SCCL) carry aberrant chromosome 3 with a common deletion of 3p14-p23. Also, Sherwin et al. found no expression of EGF receptors in these SCCL cells. To further examine the significance of these findings in SCCL development we have analyzed a new SCCL cell line, Lu-134, for its chromosome constitution and EGF binding ability. Chromosome analysis using G-banding showed that Lu-134 cell line consists of two types of cells, hypotriploid and hypotetraploid. Both cell types had a pair of apparently normal chromosome 3. Instead of abnormality in chromosome 3, the analysis using high resolution banding revealed an abnormal chromosome 2 (2q-) and several other marker chromosomes. The 2q- chromosome was found in both cell types, indicating their common derivation. The Lu-134 cell line exhibited no ability to bind [I]EGF. There was no intracellular proteins that immunoreact with antibody against human EGF receptors. The culture spent of this cell line did not inhibit [I]EGF binding to normal cells. The lack of EGF receptor expression together with other pathological evidence support the SCCL nature of Lu-134. Thus, a deletion of chromosome 3 may not be common to SCCL cells. (Supported in part by NIH grant GM-24375 and Grant-in-Aid from the Ministry of Welfare, Japan).

1212

ACTIVATION OF THE K-RAS ONCOGENE IN CHEMICALLY INDUCED RAT LUNG TUMORS, Shari J. Stowers, Steven H. Reynolds, Marshall W. Anderson, Robert R. Maronpot and *Stuart A. Aaronson, The National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 and *The National Cancer Institute, Bethesda, MD 20205

In this study, rats were exposed to the chemical, tetranitromethane (TNM) by inhalation at their maximum tolerated dose. This resulted in three types of lung tumors. High molecular weight DNA was isolated from these tumors and was transfected into NIH/3T3 mouse fibroblasts. Fourteen out of nineteen samples (74%) induced morphological transformation of NIH/3T3 fibroblasts in this assay while normal rat lung tissue did not. Southern blot analysis of secondary transfectants showed that the transforming properties of the rat tumor DNA were due to the transfer of an activated cellular homolog of the K-ras oncogene. Radioimmunoprecipitation and SDS-polyacrylamide gel electrophoresis showed that the ras proteins from these secondary transfectants did not have altered mobility as has been reported previously for some activated ras proteins. Because a structural mutation could not be shown in the transfected K-ras gene product, dot blots were performed on the original tumor DNA and showed no amplification of the normal K-ras gene. This study shows reproducible activation of the K-ras oncogene by the chemical, TNM, in three different types of lung tumors in rats. Further studies are currently being pursued to elucidate the nature of the activating lesion in the transfected K-ras oncogenes.

1213 ANALYSIS OF THE SIZE AND HETEROGENEITY OF DOUBLE MINUTE CHROMOSOMES WITH PULSED FIELD GRADIENT GEL ELECTROPHORESIS; Alexander M. van der Bliek, André Bernards and Piet Borst, Nederlands Kanker Instituut, Amsterdam, The Netherlands.

To date the size and structure of double minute chromosomes have defied analysis, because they are at the lower range of resolution of light microscopy and too large for separation with conventional techniques. Through the lysis of cells and deproteinization in agarose, chromosomes remain intact and can be studied with pulsed field gradient gel electrophoresis (PFG)(Schwartz, D.C. & Cantor, C.R. (1984), Cell 37, 67). This technique permits separation of DNA up to 2000 kb. Preliminary experiments using among others COLO-320 with amplified c-myc and EL4/8 with amplified DHFR indicate that the double minutes do not move in PFG gels. However, large fragments can be obtained by digestion in agarose. Partial digestion with infrequently cutting restriction enzymes (Not I and Sfi I) shows that the double minutes first migrate in a band corresponding to 2000 kb or larger. This band is peculiar to PFG gels and represents a cut-off in size; i.e. comigration of larger molecules. The size and heterogeneity of the amplified units in double minutes are currently being investigated with various combinations of restriction enzymes and cell-lines.

1214 SPANTIDE INHIBITS BINDING AND MITOGENESIS OF THE C-TERMINAL TETRODECAPEPTIDE OF GASTRIN RELEASING PEPTIDE IN NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS, J.C. Willey*, C.E. Moser*, and C.C. Harris*; *Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD 20205

Bombesin-like peptides are autocrine factors for some small cell carcinoma of the lung cell lines. We have reported that bombesin and its mammalian equivalent, the C-terminal tetradecapeptide of gastrin releasing peptide (GRP_{14-27}) are mitogenic for normal human bronchial epithelial (NHBE) cells, and that NHBE cells possess high affinity cell-surface receptors for GRP_{14-27}. Recently, spantide, an analogue of substance P, was reported to specifically antagonize bombesin binding and trophic action in isolated pancreatic islets. In an effort to determine whether spantide might antagonize the effects of GRP_{14-27} in NHBE cells, were measured the clonal growth rate (CGR) of cells incubated in a defined medium containing 0.8 nm EGF (Willey, J.C. et al. 1984, Exp. Cell Res.) , varying concentrations of GRP_{14-27} and either none, 1 nM, 10 nM, or 100 nM spantide. In addition, we conducted GRP_{14-27} cell-surface binding assays in the absence and in the presence of varying concentrations of spantide. GRP_{14-27} increased the clonal growth rate from 0.65 ± 0.3 PD/D to a maximum of 0.88 ± 0.4 PD/D in the presence of 300 nM GRP_{14-27}. The CGR induced by 300 nM GRP_{14-27} was decreased to 0.75 ± 0.33 PD/D, 0.71 PD/D and 0.64 PD/D in the presence of 1, 10, and 100 nM spantide respectively. Spantide competed for the binding of ^{125}I- GRP_{14-27} with a K_I of 1 nM. Thus spantide specifically inhibits GRP_{14-27} cell surface binding and induction of growth in NHBE cells.

1215 ACTIVATION OF THE CARCINOGENIC EFFECT OF A CLONED HUMAN DNA SEQUENCE BY AFLATOXIN B_1 BINDING. Stringner S. Yang[1], Rama Modali[1], Janet V. Taub[1], and George C. Yang[2]. Laboratory of Cellular Oncology[1], NCI, NIH, Bethesda, MD. 20205 and Division of Chemistry & Physics[2], FDA Washingtion, D.C. 20204.

Aflatoxin B_1 (AFB_1), a highly carcinogenic metabolite of Aspergillus flavus, is well known for its potency in hepatocarcinogenesis in various animal species. AFB_1 has also been implicated in the etiology of human liver cancer (1). The molecular mechanism in which AFB_1 induces the process of hepatocarcinogenesis has yet to be understood. In this study we examined the activation of AFB_1 on a human subgenomic Hind III restricted DNA fragment molecularly cloned in Puc 8 and cultured in JM 83 cells. The origin of this 3.1 kb DNA is human hepatocellular carcinoma (hHC) cell (Mahlavu). High molecular weight (HMW) DNA from Mahlavu hHC had no DNA-mediated cell transformation capability. Upon AFB_1 activation at an optimal dossage, hHC DNA was able to induce measurable focal transformation in NIH/3T3 cell (4.8 FFU/10 ug AFB_1-DNA/1.97 pmole AFB_1). AFB_1 activation was not obtained with normal human liver HMW DNA. The 3.1 kb Hind III hHC DNA fragment, which was also found to be a preferred target of AFB_1 binding, was able to transform NIH/3T3 cells 100 times more efficiently when activated by AFB_1 at optimal range (18-32 FFU/ 200 ng DNA/22-32 femtomole of AFB_1) in a dose-effect study. We are currently examining the possibility that a mutation was induced by AFB_1 binding in in vitro activation system. (1) Autrup et. al. 1982 Carcinogenesis 3: 391-395.

Lung Cancer

1216 THE ROLE OF ONCOGENES IN HUMAN RESPIRATORY CARCINOGENESIS, Curtis C. Harris, George H. Yoakum, John F. Lechner, Paul A. Amstad, George E. Mark, Brenda I. Gerwin, and Benjamin F. Trump*, Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, Maryland 20205, and Department of Pathology*, University of Maryland School of Medicine, Baltimore, Maryland 21201

Respiratory carcinogenesis is considered to be a multistage process involving both genetic lesions and selective clonal expansion of preneoplastic and neoplastic cells. Putative activation of oncogenes has been identified in human lung cancers by isolating human tumor DNA sequences that transform murine NIH 3T3 cells and by measuring increased levels of oncogene mRNA in both primary tumors and tumor cell lines. Three multigene families of oncogenes, i.e., ras, myc, and raf, have been so far associated with lung cancer.

We are investigating the role of these and other oncogenes in the malignant transformation of normal human bronchial epithelial (NHBE) cells in vitro. Our previous studies have shown that NHBE cells can be successfully grown in serum-free culture medium for 20-40 population doublings before senescence and are induced to undergo terminal squamous differentiation by suspension in semisolid media, blood-derived serum, tumor promoters and high cell density culture conditions. In contrast, human lung cancer cell lines continue to grow under these conditions and thus have a survival advantage. We are using these selective clonal expansion pressures to isolate phenotypically altered cells following oncogene transfection of NHBE cells using the protoplast fusion method. Using this strategy, several clones of HBE cells transfected with vH-ras oncogene have been isolated. The vH-ras oncogene has been stably integrated into the cellular DNA and both mRNA and P21 gene product of this oncogene can be measured in these clones. One of these clones, TBE-1, has been extensively characterized and has the following properties: (1)grows for more than 120 population doublings and is apparently immortal; (2)forms multilayered foci and has a higher saturation density than NHBE cells; (3) is resistant to induction of terminal squamous differentiation by high concentration (100 nM) of 12-O-tetradecanoyl-phorbol-13-acetate; (4) is aneuploid; (5) produces the "ectopic" hormone, beta human chorionic gonadotropin; and (6) TBE-1 cells cloned in semisolid medium form persistent, anaplastic tumors when xenotransplanted into athymic nude mice. These results and those from other studies suggest that oncogenes can play a role in the malignant transformation of human epithelial cells.

1217 **TUMORIGENIC AGENTS IN TOBACCO PRODUCTS AND THEIR UPTAKE BY CHEWERS, SMOKERS AND NONSMOKERS.**

Dietrich Hoffmann, Stephen S. Hecht, Nancy J. Haley, Klaus D. Brunnemann, John D. Adams, and Ernst L. Wynder. American Health Foundation, Valhalla, New York, 10595.

The presentation will review the chemical nature and formation of tumor initiators, tumor promoters, cocarcinogens and organ-specific carcinogens in tobacco smoke. Of special significance are the tobacco-specific N-nitrosamines (TSNA) which are formed from nicotine and which are powerful carcinogens, inducing tumors of the upper digestive tract and the upper respiratory tract of mice, rats and hamsters. One of these carcinogens, NNK, induces also squamous cell carcinoma and adenocarcinoma in the lungs of rats. TSNA are not only present in chewing tobacco, snuff and tobacco smoke but are most likely also formed endogenously during chewing and upon smoke inhalation.

Since most low-yield cigarettes deliver also lesser amounts of nicotine, the major habituating agent in tobacco products, smokers alter their smoking intensity in order to reach a certain nicotine bolus. This compensatory behavior will be substantiated by data from biochemical measurements and the endogenous formation of nitrosamines in chewers and smokers will be documented.

The uptake of sidestream tobacco smoke constituents by nonsmokers will be demonstrated in controlled polluted environments and in field studies with adults and with infants of smoking mothers.

Our studies in tobacco carcinogenesis are supported by Grants CA-2193, CA-29580 and CA-35607 from the National Cancer Institute.

1218 GROWTH FACTORS AND ONCOGENES IN HUMAN LUNG CANCER CELLS, John D. Minna, Marion M. Nau, Frank Cuttitta, James Battey, Jay Brooks, Desmond N. Carney, Martin Brower, Herbert Oie, Bruce Johnson, Adi F. Gazdar, NCI-Navy Medical Oncology Branch, National Cancer Institute, Bethesda, MD 20814.

Advances in the ability to grow lung cancer in vitro have come following the definition of serum free hormone supplemented media for different lung cancer types. A large panel of lung cancer cell lines has been generated using this and other methods. In addition, we have found that SCLC produces a regulatory peptide related to amphibian bombesin (mammalian equivalent is gastrin releasing peptide, GRP). The cells also have high affinity receptors for GRP and their clonal growth is stimulated by GRP in serum free medium. All of these findings suggest that bombesin/GRP function as an autocrine growth factor for SCLC. This was confirmed by preparing a monoclonal antibody against bombesin which reacts with the c-terminal portion. This antibody blocks the binding of bombesin/GRP to its cell surface receptor, inhibits the growth of SCLC in vitro and in vivo in nude mouse xenografts. Anti-idiotypic antibodies prepared against this monoclonal also appear to bind to the cell surface receptors for bombesin/GRP. Whether bombesin/GRP or their receptors are related to known oncogenes is under study.

Recently, we have found that a large number of SCLC have amplification of one other of the myc family of oncogenes. SCLC with c-myc amplification produce large amounts of c-myc mRNA and have a different phenotype than SCLC without c-myc amplification including shorter doubling time, higher cloning efficiency, faster xenograft growth, decreased expression of differentiated functions, and increased radio-resistance. Transfecting the c-myc gene into a SCLC that is not amplified for c-myc results in some of these altered features. We have also found the N-myc proto-oncogene to be amplified and greatly expressed in SCLC. In addition, using N-myc probes we have identified, cloned, and begun characterization on a new member of the myc family which we term L-myc. This gene has regions of great nucleic acid and amino acid sequence homology with c-myc and N-myc but is quite distinct from these other two genes. Altogether nearly 2/3rds of SCLC have amplification and/or greatly increased expression of one of these three myc family members. Early clinical correlations suggest that patients with c-myc and probably N-myc amplification have impaired survival compared to patients without these amplifications.

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1219 **THE CHANGING EPIDEMIOLOGY OF LUNG CANCER**

Ernst L. Wynder, Marc T. Goodman, Geoffrey C. Kabat, and Ian T.T. Higgins. American Health Foundation, New York, New York, 10017.

Current lung cancer incidence patterns reflect changes in the consumption as well as in the make-up of cigarettes that have occurred over the past three decades and are especially indicative of the etiologic significance of increased cigarette smoking among women.

At the same time, evaluation of larger data bases in retrospective studies allows for verification of histologic patterns of lung cancer in men and women in respect to tobacco usage and inhalation practices. Furthermore, epidemiologic surveys now consider the role of nutrients and micronutrients of the host in the induction and development of lung cancer. Epidemiologic and experimental evidence of synergistic and cocarcinogenic agents involved in the pathogenesis of lung cancer in "high risk groups" will be reviewed.

Latency of lung cancer occurrence in regard to onset, duration and cessation of smoking habits will be examined, especially as it relates to models of tumor promotion.

The weaker associations of etiologic agents such as environmental tobacco smoke and automotive emissions with lung cancer incidence will be discussed.

Our studies are supported by Grant 1PO-CA-32617 from the National Cancer Institute.

Cancer-Prone Diseases and DNA Repair

1220 NEW MODEL FOR PYRIMIDINE DIMER REPAIR IN HUMAN CELLS, M. Paterson, N. Gentner, M. Middlestadt and M. Weinfeld, Chalk River Nuclear Laboratories, Chalk River, Ontario

A re-examination of the metabolic fate of UV-induced cyclobutyl pyrimidine dimers in dermal fibroblasts from patients with different genetic forms of xeroderma pigmentosum (XP), a rare cancer-prone skin disorder, has provided new insight into the mode of dimer repair in normal cells. When DNA extracted from post-UV (254 nm; 15 $J \cdot m^{-2}$) incubated cultures was subjected to enzymatic photoreactivation (PR) to probe dimer authenticity, single-strand scissions were produced in the damaged DNA of incubated XP group A or D cells, but not in DNA from XP variants or normal cells. Since enzymatic PR splits only the cyclobutane ring, our results suggest that in dimer excision-defective XP A and D strains, the intradimer phosphodiester bond may be cleaved without site restoration. That such cleavage may be an early step in the normal excision-repair process was indicated by the observed release of free thymidine (dT) and thymidine monophosphate (dTMP) (but not thymine) upon photochemical reversal of the dimer-containing excision fragments isolated from post-UV incubated normal cells. The combined number of dT and dTMP molecules released was equal to 50-70% of the number of dimers photoreversed; for such reversal to occur, the dimer must both be at one end of an excised fragment and contain an internal phosphodiester break. We therefore propose a new model for dimer repair in human cells in which hydrolysis of the intradimer phosphodiester linkage precedes the concerted action of a generalized "bulky lesion-repair complex" involving conventional strand incision/lesion excision/repair resynthesis/strand ligation reactions.
[Supported in part by US NCI Contract NO1-CP-21029 (Basic)]

Carcinogen Metabolism and DNA Damage

1221 METABOLIC ACTIVATION OF MUTAGENIC AND CARCINOGENIC DINITROPYRENES. Frederick A. Beland, Zora Djuric, E. Kim Fifer and Robert H. Heflich, Department of Biochemistry, University of Arkansas for Medical Sciences, Little Rock, AR 72205 and National Center for Toxicological Research, Jefferson, AR 72079.

Dinitropyrenes, environmental contaminants found in diesel emission, are carcinogenic in rats and mice, and are among the most mutagenic compounds ever tested in the Salmonella reversion assay. Incubation of S. typhimurium TA1538 with 1,8-dinitropyrene resulted in the formation of one major DNA adduct, N-(deoxyguanosin-8-yl)-1-amino-8-nitropyrene. The same adduct was formed when the reduced intermediate, N-hydroxy-1-amino-8-nitropyrene, was reacted with DNA, thus suggesting that nitroreduction was involved in the metabolic activation of this dinitropyrene. Although 1,8-dinitropyrene was at least 40-fold more mutagenic than 1-nitropyrene, the number of reversions per adduct induced by the C8-substituted deoxyguanosine adducts of either 1-nitropyrene or 1,8-dinitropyrene was similar. 1,8-Dinitropyrene was more mutagenic than 1-nitropyrene because N-hydroxy-1-amino-8-nitropyrene served as a substrate for a bacterial transacetylase and therefore bound to DNA more efficiently than did N-hydroxy-1-aminopyrene, which did not appear to be a substrate for this enzyme. Similar routes of metabolic activation occurred with rat liver cytosol; 1,8-dinitropyrene was reduced to a metabolite which was further activated by acetyl coenzyme A-dependent transacetylation while 1-nitropyrene was not. These data indicate that nitrated pyrenes are metabolized to genotoxic products by nitroreduction and that 1,8-dinitropyrene is further activated by O-esterification.

1222 EXTRACHROMOSOMAL PROBES FOR MUTAGENESIS BY CHEMICAL CARCINOGENS, J.M. Essigmann, C.L. Green and E.L. Loechler. Massachusetts Institute of Technology, Cambridge, MA 02139

An important step in chemical carcinogenesis is thought to be the formation of covalent adducts between DNA and reactive forms of chemical carcinogens. Misreplication or misrepair of chemically modified DNA causes mutations, which in principle could initiate malignant transformation of somatic cells. Our approach for establishing the relationship between adduct structure and biological effects is to situate individual adducts at defined sites in genomes, allow enzymatic processing to occur in vivo, and then assess the resulting genetic change(s) both qualitatively and quantitatively. This work examines the mutagenic activity of O^6-methylguanine (O^6MeGua), a DNA adduct formed by certain carcinogenic alkylating agents. A tetranucleotide, 5'-$T_p m^6G_p C_p A$-3', was synthesized by the phosphotriester method and ligated into a four-base gap in the unique Pst I site of the duplex genome of the E. coli virus, M13mp8. The double-stranded ligation product was converted to single-stranded form and used to transform E. coli to produce progeny phage. The mutation frequency of O^6MeGua was defined as the percentage of progeny phage with mutations in their Pst I site, and this value was determined to be 0.4%. To determine the impact of DNA repair on mutagenesis, cellular levels of O^6MeGua-DNA methyltransferase (an O^6MeGua-repair protein) were depleted by treatment of host cells for virus replication with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) prior to viral DNA uptake. In these host cells, the mutation frequency due to O^6MeGua increased markedly with increasing MNNG dose (the highest mutation frequency observed was 30%). DNA sequence analysis of mutant genomes revealed that in both MNNG treated and untreated cells, O^6MeGua induced exclusively G to A transitions.

1223 STEREOCHEMICAL AND ELECTRONIC DETERMINANTS OF THE MUTAGENICITY AND TUMORIGENICITY OF POLYCYCLIC HYDROCARBON BAY-REGION DIOL EPOXIDES. A.W. Wood, W. Levin, R.L. Chang, R.E. Lehr, D.R. Thakker, H. Yagi, J.M. Sayer, D.M. Jerina and A.H. Conney, Roche Res. Center, Nutley, NJ 07110, U. Oklahoma, Norman, OK 73019 and NIADDK, NIH, Bethesda, MD 20205.

Bay-region diol epoxides, ultimate carcinogenic metabolites of a number of polycyclic hydrocarbons, exist as diastereomeric pairs of optical isomers. Studies with a large number of diol epoxides, including five complete sets of optical isomers, have elucidated critical determinants of biological activity. Among sterically unhindered bay-region diol epoxides, only the isomer with (R,S,S,R) absolute configuration is exceptionally tumorigenic to mice, or exceptionally mutagenic to cultured mammalian cells. The mutagenicity of these (R,S,S,R) isomers in both bacterial and mammalian cells shows an excellent correlation ($r \geq 0.98$) with observed chemical reactivity (spontaneous hydrolysis) or predicted carbocation resonance stabilization. However, steric factors, as in the case of the diol epoxide isomers of benzo[c]phenanthrene, or electronic factors, as in the case of 6-flurobenzo[a]pyrene, can induce conformational changes in the hydroxyl groups of diol epoxides that result in marked changes in biological activity. Introduction of a nitrogen hetero-atom into a polycyclic hydrocarbon can also change the electronic properties of the diol epoxides altering both mutagenic and tumorigenic activity. Thus, the tumorigenic activity of polycyclic hydrocarbons is determined not only by the extent of metabolic formation and detoxification of the bay-region diol epoxides, but by the relative and absolute configuration of their hydroxyl and epoxide moieties, the conformation of the hydroxyl groups, and the extent to which resonance stabilization facilitates carbocation formation.

DNA Damage and Repair; Environmental and Chemical Carcinogenesis

1224 STABILITIES AND MUTAGENIC CONCENTRATIONS OF CHEMICAL CARCINOGENS IN A DIFFUSION BIOASSAY USING DIFFERENT STRAINS OF s. typhimurium, Tamara E. Awerbuch and Anthony J. Sinskey, Harvard School of Public Health, Boston, MA 02115 and MIT, Cambridge, MA 02139

The mathematical model describing a diffusion bioassay was based on the assumption that the mutagenic action of the diffusing chemcial from the center of a dish (containing a bacterial lawn in an agar gel) is solely related to the time-space concentration of the diffusing substance and is given by the equation: $\partial c/\partial t = D(\partial^2 c/\partial t^2 + 1/r\ \partial c/\partial r) - c/\tau$; there is no term related to the microorganism in the system. r is the distance from the center of the dish, t is diffusion time, D is the diffusion coefficient and τ is the decay time. The central results are: 1) an equation for an effective time-integrated concentration distribution $C(r)$. From knowledge of the radius r_{mut} the lower limit C_{mut} at which mutagenicity occurs can be readily calculated. 2) an equation for determining τ using experimental data from the diffusion bioassay: $\ln c_o = r_{mut}/\sqrt{D\tau} + \text{const.}$ c_o is the initial concentration of the chemical at the center of the dish. In this work we tested the validity of the assumption with different strains of s. typhimurium. The half-life ($\tau_{1/2}$) of N-methyl N'-nitro-N-nitrosoguanidine (NG) was calculated by the diffusion assay, using strains hisG46, TA1950, TA1535 and TA100. For all strains $\tau_{1/2}$ equalled 2.2h; strain sensitivity for detecting threshold mutagenic concentrations of NG was essentially the same, except that hisG46 was slightly more sensitive.

1225 POTENTIATION OF ALKYLATING AGENT-INDUCED CELL KILLING BY 3-AMINOBENZAMIDE IN MNNG-RESISTANT AND -SENSITIVE HUMAN CELLS, Michael A. Babich and Rufus S. Day III, Nucleic Acids Section, LMC, DCE, National Cancer Institute, Bethesda, MD 20205.

The inhibitor of poly(ADP-ribose) synthesis 3-aminobenzamide (3AB) is known to inhibit the recovery of L1210 cells exposed to alkylating agents. Here we show the effect of 3AB on the colony forming ability of human cells exposed to alkylating agents. In the presence of 3 mM 3AB, MNNG-resistant fibroblasts KD were 16 times more sensitive to MMS in the pre-shoulder region (0-1.5 mM MMS) of the survival curve than control cells. That is, for every "lethal hit" incurred by control KD cells at a given MMS dose, 3AB-treated cells incurred 16 lethal hits. At MMS doses <1 mM, 10 mM 3AB-treated KD cells were 50 times more sensitive to MMS; 90% of the 3AB-potentiable damage was removed from control cells within 24 hours of MMS treatment. KD cells treated with 5 mM 3AB were six times more sensitive to MNNG than controls, but were NOT more sensitive to CNU. However, MNNG-sensitive, O(6)-methylguanine methyltransferase-deficient cells GM638 and IMR90-830 treated with 3mM 3AB were only about twice as sensitive to MMS as controls and only two to four times more sensitive to MNNG. In MNNG-sensitive, O(6)-methylguanine methyltransferase-proficient A498 cells, MMS-induced lethality was NOT potentiated by 3 mM 3AB, while MNNG-induced lethality was potentiated two-fold by 5 mM 3AB. We conclude that: (1) Potentiation of lethality by 3AB is greatest in MNNG-resistant cells; (2) The relative number of 3AB-potentiable lesions depends on the alkylating agent in the order--MMS > MNNG >> CNU; and (3) The 3AB-potentiable lesions are probably DNA bases with alkyl groups on nitrogen, rather than oxygen, atoms.

1226 ALKYLATION AND REPAIR OF DNA IN LUNG AND LIVER FOLLOWING ADMINISTRATION OF THE TOBACCO SPECIFIC CARCINOGEN 4-(N-METHYL-N-NITROSAMINE)-1-(3-PYRIDYL)-1-BUTANONE (NNK), Steven A. Beljnsky[a], Felix Romagna[a], Frank C. Richardson[b], James A. Swenberg[b], and Marshall W. Anderson[a], The National Institute of Environmental Health Sciences[a] and Chemical Industry Institute of Toxicology[b], Research Triangle Park, NC 27709

NNK, one of the major nitrosamines detected in tobacco smoke, has been found to induce a high incidence of lung, liver, and nasal cavity tumors in laboratory animals. The formation and removal of the promutagenic lesion O^6-methylguanine (O^6MG) was determined over 12 days of NNK administration (100 mg/kg,i.p./day) to Fischer 344 rats. O^6MG levels were maximal in hepatocytes after 2 days of carcinogen treatment and declined rapidly during the remaining 10 days. In contrast, O^6MG levels increased in the lung throughout the 12 days of carcinogen treatment indicating a limited capacity for repair of this lesion. Steady-state levels of 7-methylguanine (7MG) were observed in both liver and lung after 2 to 4 days of treatment with NNK. The ratios of O^6 to 7MG alkylation were also similiar in both tissues following 2 days of carcinogen treatment. However, after 12 days, a 14-fold decrease and a 2-fold increase in this ratio was observed in hepatocytes and lung, respectively. Thus, repeated exposure to NNK through heavy smoking could lead to an accumulation of methylated DNA adducts, which in turn may be an important contributing factor to the association between tobacco use and cancers of the respiratory system (ES05292).

1227 STRAIN SPECIFICITY IN "IN VITRO" AND "IN VIVO" ACTIVATION OF THE ORGANSPECIFIC CARCINOGEN DMH(1,2-DIMETHYLHYDRAZINE). Lidia C. Boffa and Claudia Bolognesi, Istituto Nazionale per la Ricerca sul Cancro,IST, Viale Benedetto XV n.10, 16132 Genova,Italy

The methylating carcinogen DMH is ,under certain experimental conditions, organspecific and usually produces adenocarcinoma of the colon in rodents with high efficency. However a differential susceptibility to the carcinogen has been described for various mice strains:SWR/J mice are highly susceptible while AKR/J are resistant.
As from our recently reported data on the rat we have now indications that also in the mouse colon microsomes could be one of the modulating factors in genotoxicity and probably organspecificity of DMH. The ability of the colon microsomes from one specific mouse strain to produce cytotoxic metabolite(s) follows the same trend than teir susceptibility to the carcinogen itself: AKR/J but not SWR/J colon microsomes are able to produce "in vitro" DMH metabolite(s) more toxic and mutagenic than the parent compound. The mutagenic potency of these compounds was judged by several criteria: 1)autoradiographic identification of unscheduled DNA synthesis 2)induction of 6-thioguanine resistant mutants in V79 Chinese hamster cells 3) formation of adducts of DNA bases (in particular O^6-methylguanine) 4)evaluation of DNA damage by alkaline elution technique.
The results of these experiments tend to confirm that colon microsomes are probably involved in the strain dependent organspecificity of DMH carcinogenesis. We would like also to advance the hypothesis of a similar mechanism in the genetically dependant human susceptibility to colon cancer .

1228 DNA DAMAGE AND REPAIR IN A SPECIFIC, ACTIVE GENE IN MAMMALIAN CELLS. Vilhelm A. Bohr, Charles A. Smith, Isabel Mellon, Diane Okumoto and Philip C. Hanawalt, Department of Biological Sciences, Stanford Univ., Stanford, CA 94305.

We have studied the formation and repair of pyrimidine dimers in the dihydrofolate reductase (DHFR) gene of CHO cells amplified for the gene. Restriction fragments inside and outside the transcribing unit of the gene were analyzed for dimer content by use of a dimer specific endonuclease and Southern transfer and hybridization following alkaline gel electrophoresis. Two thirds of the dimers produced by 20 J/m^2 were removed from a fragment within the gene in 24 hours. In contrast, only 10% were removed from fragments upstream within the amplified unit, as well as from the genome overall. Preliminary results with non-amplified CHO cells also indicate efficient repair of the gene. Preferential repair of the DHFR gene in amplified cells was confirmed by the use of a monoclonal antibody to BrUra-substituted DNA. Unreplicated restricted BrUra-containing DNA was precipitated with the antibody to allow separate analysis for DHFR sequences of restriction fragments with and without repair patches. Efficient repair of essential regions of the genome might explain why these rodent cells survive UV damage as well as human cells, but remove far fewer dimers from their genomes overall. Our experiments also provide information about the effects of DNA damage on replication of specific sequences. Initial results suggest an inhibition of replication of the DHFR sequences during the repair period.
We are currently analyzing repair and replication in human cells in a similar manner.

1229 CC SITES WITH ELEVATED UV MUTATIONS IN dcm⁻ E. coli HAVE ELEVATED (6-4) LESIONS BUT NOT ELEVATED CYCLOBUTANE PYRIMIDINE DIMERS, Douglas E. Brash, Roeland Schaaper, William A. Haseltine, and Barry Glickman, National Cancer Institute, Bethesda, MD 20205, National Institute of Environmental Health Sciences, Research Triangle Park, NC, and Dana-Farber Cancer Institute, Boston, MA 02115

Dcm⁻ E. coli do not methylate the 3' cytosine of $CC^{A}/_{T}GG$ sequences in the lacI gene. We find that the UV-induced mutation frequency at such sites is elevated in dcm⁻ compared to wild-type. The frequency of UV-induced pyrimidine-pyrimidone (6-4) photoproducts is elevated in dcm⁻ at these sites, but cyclobutane pyrimidine dimers are not.

These results suggest that at these sequences in wild-type E. coli, the UV premutagenic lesion is not the cyclobutane pyrimidine dimer and is the pyrimidine-pyrimidone (6-4) photoproduct.

1230 **MOLECULAR MECHANISMS OF CHEMICAL CARCINOGENESIS,** R.W. Chambers, R.G. Fenwick, S.W. Cline, S.S. Tsang and L. Ireland, Dalhousie University, Halifax, Nova Scotia B3H 4H7 We are developing a site-specific mutagenesis system to study the carcinogenic activity of single, well characterized, covalent adducts that represent products known to form when specific carcinogens react with DNA in vivo. Our approach is based on the bacteriophage system we have developed for studying the molecular mechanisms of mutation by carcinogens. The carcinogen adduct to be studied (e.g. O^6-methyl guanine) is introduced into an oligonucleotide of preselected sequence by chemical synthesis and incorporated into biologically active DNA enzymatically. The mutagenic activity of the adduct is then examined using suitable test cells. For our carcinogenesis studies we have recloned the human Ha-ras oncogene, T24, and its protooncogene counterpart into M13mp10. From viral clones, we have isolated single stranded DNA corresponding to the + and - strands of these 2 genes. We have constructed the 4 possible partial duplexes by hybridizing the appropriate strands and shown that onc/onc, ponc/onc and onc/ponc, but not ponc/ponc transform NIH/3T3 cells. These ss DNA's provide the starting material for positioning site-specific adducts in either the 12th or 61st codon. We have constructed a DNA that transforms NIH/3T3 cells by enzymatic elongation of an oligonucleotide carrying the 12th codon of the ras oncogene on a protooncogene template, cutting the crude products with XhoI + ClaI, and isolating the 5392 restriction fragment. We are currently using this method to study the transforming activity of several alkyl adducts that are known products when alkyl nitroso compounds react with DNA in vivo. Similar studies are under way with site-specific modifications located in the 61st codon. This work was supported by the National Cancer Institute of Canada.

1231 REPLICATIVE AND REPAIR DNA LIGASE ACTIVITIES DURING HEPATOCARCINOGENESIS, John Y. H. Chan and Frederick F. Becker, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030

A progressive accumulation of DNA breaks has been reported to occur in nuclear DNA obtained from putative premalignant hepatic lesions induced by carcinogens. To determine if this alteration resulted from a defect in ligation, DNA ligases were compared in normal rat liver, 24-h regenerating liver and in hepatic nodules at intervals after cessation of N-2-acetylaminofluorene (AAF) treatment. Multiple forms of nuclear DNA ligase activity were detected by gel filtration chromatography. The activities of the replicative species, DNA ligase Ia (480 kd) and DNA ligase Ib (240 Kd), were increased 4-fold and 2-fold above normal rat liver, respectively, in 24-h regenerating livers, while in AAF-induced nodules, these species were increased 3-fold and 1.5-fold. DNA ligase II (80 Kd) the repair enzyme, was found in both soluble nuclear fractions and chromatin at approximately identical levels in all tissues tested. The various ligase demonstrated identical responses to salt and heat in all tissues. The failure to detect significant alterations in activity of ligases in the hepatic nodules or in their biochemical characteristics indicate that the accumulation of DNA damage (presumably breaks) is not due to an alteration in level or the biochemical properties of DNA ligases. However, experiments on the restriction of template activities and endonuclease sensitivities indicate that conformational changes in nodular chromatin may be the cause for the accumulated DNA breaks. Supported by NIH grant CA 20657.

1232 TRANSFER OF CHINESE HAMSTER DNA REPAIR GENE(S) INTO UV-SENSITIVE REPAIR-DEFICIENT HUMAN CELLS (XP), James E. Cleaver and Deneb Karentz, Laboratory of Radiobiology, University of California, San Francisco, CA 94143

Xeroderma pigmentosum (XP) is an inherited human disease which is caused by the inability of cells to repair UV-damage to DNA. There are at least 9 complementation groups in XP and many similar groups in Chinese hamster ovary (CHO) cells. Thus far, cloning repair genes by genomic transfection has failed in XP cells. We have designed experiments to transfer whole chromosomal fragments from repair competent CHO cells into XP cells in order to identify DNA sequences from CHO cells which function in repairing DNA of XP cells. X-irradiated CHO cells were fused to DNA repair-deficient human fibroblasts (XP12RO) using polyethylene glycol. UV-resistant hybrids were selected by periodic exposures to UV light. Several secondary and tertiary hybrids have been selected with progressively lower amounts of CHO DNA. Hybrid cell lines have slightly higher chromosome numbers than XP12RO cells. DNA-DNA hybridizations indicate that these hybrids contain varying proportions of CHO DNA and are not XP12RO revertants. The hybrid lines exhibit resistance to UV that is comparable to that of CHO cells and they are also proficient at repair replication after UV exposure. DNA hybridization techniques are being applied to identify hamster repeated sequences which have become integrated into the hybrid genomes. It is expected that hamster genes coding for repair enzymes or other UV-resistant processes can be cloned from these XP12RO-CHO hybrids. Work supported by U.S. Department of Energy and National Institute of Environmental Health and Safety.

1233 REPAIR OF O^6-METHYLGUANINE IN VARIOUS HUMAN CELL TYPES, Steven M. D'Ambrosio, Gulzar Wani, Ruth E. Gibson-D'Ambrosio and Altaf A. Wani, Ohio State University, Columbus, Ohio 43210.

The persistance of the O^6-alkylguanine lesion in the DNA of certain animal tissues has been implicated in the carcinogenic effect of nitrosoureas. However, comparatively little is known about the effects of nitrosoureas and the relationship of DNA lesions to the induction of human cancers. In order to better understand how various human tissue and cell types respond to carcinogenic insult, we developed procedures for culturing human fetal epithelial (intestine and kidney), fibroblast (skin), glial and neuronal (brain) cell types. Using these cell cultures , we characterized the cellular kinetics for the repair of O^6-methylguanine. Cells were treated with 150 uM [^{3}H]methylnitrosourea for 30 min. At 0 to 4 hr post treatment, the DNA was extracted, acid hydrolyzed and the methylated bases separated by hplc. Cells derived from all 4 tissues are proficient for the repair of O^6-methylguanine. The kinetics for repair appears to be biphasic with an initial rapid phase followed by a slower phase. The half-life for removal of this lesion is 30 (brain, skin) to 60 (kidney, intestine) min. Four hours post treatment, 65%, 67%, 75% and 80% of the O^6-methylguanine was repaired in kidney, intestine, skin and brain derived cells respectively. During this period, <15% of the N7-methylguanine was removed from the DNA of all cell types. Supported by grants from the NIEHS (ES3101) and USEPA (R810146).

1234 ONCOGENES, INTERFERON, AND DNA REPAIR, Rufus S. Day, III *, Takashi Yagi*, and Carole Heilman†, Laboratory of Molecular Carcinogenesis* and Laboratory of Experimental Carcinogenesis†, DCE, NCI, NIH, Bethesda, MD 20205.

The RS 485 strain is a line of NIH3T3 cells transformed by the human c-Ha-ras1 oncogene activated by an LTR from Ha-MuSV (Chang, E.H., et al., Nature 297, 479, 1982). Compared to NIH3T3 cells, RS 485 is deficient in ability to repair O^6-methylguanine in DNA and is hypersensitive to inactivation by choroethylnitrosourea, (CNU), an agent that selectively kills cells lacking the ability to repair O^6-methylguanine. We have found that the interferon-produced reversion of the morphologically transformed RS 485 cells to morphologically flat colonies (Samid, D., et al., Biochem. Biophys. Res. Commun. 119, 21, 1984) is often accompanied by a recovery of DNA repair. Two of three flat colonies isolated showed both enhanced repair of O^6-methylguanine and increased resistance to killing by CNU. The third, although somewhat flatter than RS 485, has the repair defect characteristic of RS 485. Samid et al. also showed that the flat revertants produced by interferon treatment of RS 485 had decreased transcription of the Ha-ras1 gene. In preliminary studies, our data is similar to that of Samid et al. One repair-proficient flat revertant had reduced transcription of Ha-ras1, whereas the repair-deficient revertant did not.

In earlier studies of human cells, transformation of each of three human fibroblast strains by SV40 was accompanied by loss of DNA repair (Day, et al. Nature 288, 724, 1980). In the case of transformation of NIH3T3 cells by the c-Ha-ras1 gene such transformation-linked shutoff of DNA repair appears closely linked to expression of the oncogene.

1235 EFFECTS OF UV LESIONS UPON SV40 REPLICATION FORKS, Howard J. Edenberg and Cheryl A. Berger, Dept. of Biochemistry, Indiana Univ. School of Medicine, Indianapolis, Indiana 46223.

To examine the mechanism by which ultraviolet photoproducts inhibit DNA replication in mammalian cells, we are studying the replication of Simian Virus 40 (SV40) in monkey cells. UV causes a rapid and dramatic reduction in the amount of labeled thymidine incorporated into SV40 DNA. The fraction of molecules able to complete replication is even more severely depressed. Analyses of the molecules made after UV indicate that they are replication intermediates in which synthesis of daughter strands is blocked. The sizes of the daughter strands suggests blockage at the sites of pyrimidine dimers (see Edenberg, Virology 128, 298-309). Taken together, these data suggest that replication forks are blocked when they encounter pyrimidine dimers in the template for the continuously synthesized strand. Preliminary results from electron microscopy of replication intermediates support this interpretation.

1236 PROMOTION, ANTIPROMOTION, AND REPAIR/MISREPAIR PROCESSES IN RADIATION-INDUCED NEOPLASTIC TRANSFORMATION. *M.M.Elkind, **C.K.Hill, and ***A.Han[1], *Dept. of Radiol. & Radiat. Biol., Colo. State Univ., Ft. Collins,CO 80523; **Div. of Biol. & Med. Res., Argonne Nat. Lab., Argonne,IL 60439; ***Univ. of So. Calif., Los Angeles, CA 90015.

Using C3H mouse 10T½ cells, we have been studying the radiobiology of neoplastic transformation in vitro. Earlier results showed that protracting the exposure to a sparsely ionizing radiation, i.e., ^{60}Co γ-rays, led to a reduced frequency [A.Han et al., Cancer Res. 40, 3328 (1980); C.K.Hill et al., Carcinogenesis 5, 193, (1984)] whereas protraction of a densely ionizing radiation, i.e., fission-spectrum neutrons, increased the frequency [C. K. Hill et al., Int. J. Radiat. Biol. 46, 11 (1984)].

We now report that the phorbol ester, TPA, has a differential effect on the enhanced frequency that is observed depending upon the quality of the radiation. Although TPA increases transformation after both kinds of radiation, the magnitude of the change is characteristically different for γ-rays vs. fission-spectrum neutrons. Also, preliminary data show that Antipain can reverse the enhanced frequency due to the protraction of neutron exposures. We interpret the effect of TPA to indicate that it acts to express a late event but one whose properties depend on the quality of the radiation used. (Work supported by the U.S. Dept. of Energy, Contract No. W-31-109-ENG-38, and the U.S. Nat. Cancer Institute, Grant No. CA 29940.)

[1]Deceased, 7 May 1984.

1237 REPAIR OF UV LIGHT-INDUCED (6-4) PHOTOPRODUCTS IN HUMAN CELL LINES William A. Franklin and William A. Haseltine, Laboratory of Biochemical Pharmacology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

UV light-induced (6-4) photoproducts are DNA adducts formed between adjacent pyrimidines in a DNA strand. Although these products are less abundant than cyclobutane pyrimidine dimers, (6-4) products have been implicated as premutagenic lesions in several prokaryotic systems. In E. coli, (6-4) products are removed from the cellular DNA by the uvrABC enzymatic excision complex.

The removal of (6-4) products was measured in a series of human cell lines. HeLa cells, GM637 cells (SV40 transformed fibroblasts) could effectively remove both T<>T cyclobutane dimers and TC (6-4) products from DNA. Two xeroderma pigmentosum cell lines, M1 (SV40 transformed complementation group A fibroblasts) and GM2995 (SV40 transformed complementation group C fibroblasts) were unable to excise cyclobutane dimers, but demonstrated excision of the TC (6-4) product. A revertant cell line, XPRH10, showed efficient removal of both dimers and (6-4) photoproducts. These results suggest a possible difference in the excision pathways for these two types of UV light-induced lesions.

1238 THE MOLECULAR CLONING AND CHARACTERIZATION OF YEAST GENES REQUIRED FOR EXCISION REPAIR OF DNA - E.C. Friedberg, J. Chenevert, R. Fleer, L. Naumovski, G. Pure, G.W. Robinson, W. Weiss and E. Yang, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

By screening a yeast genomic library we have isolated recombinant DNA plasmids that complement the sensitivity to ultraviolet (UV) radiation of rad1, rad2, rad3, rad4 and rad10 mutants of S. cerevisiae. Genetic analysis following integration of the cloned yeast inserts into the genome of appropriate yeast strains demonstrated that some of the plasmids contain the yeast RAD1, RAD2, RAD3 or RAD10 genes. A plasmid that uniquely complements the ochre mutant rad4-3, contains a normal $tRNA^{Gln}$ gene that partially suppresses the ochre mutation when present in high copy number. The complete nucleotide sequence of the RAD1 and RAD3 genes have been established and the location and nature of the rad3-1 and rad3-2 mutations have been identified by sequence analysis. The RAD1 gene could encode a protein of ∿110kDa and RAD3 could encode a protein of ∿90kDa. The RAD2 gene encodes a mRNA 3.2kb in size which could yield a protein of ∿ 128kDa. The smallest subclone of RAD10 that fully complements rad10 mutants is 1.1kb. Hence the RAD10 protein may not be larger than ∿ 44kDa. The RAD3 gene is essential for the viability of haploid yeast in the absence of DNA damage, however, RAD1 and RAD2 are not essential genes. We have constructed fusions between the 5' regulatory regions of various RAD genes and the 3' region of the E. coli lac'z gene. By transforming cells with various fusion plasmids, we have established that the expression of neither RAD1 nor RAD3 is induced by DNA damage.

1239 TRANSFORMATION OF NORMAL HUMAN FIBROBLASTS WITH V-SIS VIA DNA TRANSFECTION, Dennis G. Fry, Lonnie D. Milam, Veronica M. Maher, and J. Justin McCormick, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824-1316.

The oncogene of Simian Sarcoma Virus (v-sis) 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. 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. Supported by DOE Contract 0459.

1240 PATHOBIOLOGICAL EFFECTS OF TOBACCO SMOKE CONDENSATE (TSC) FRACTIONS AND SMOKE-RELATED ALDEHYDES IN CULTURED NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS, R.C. Grafstrom*, J.C. Willey**, and C.C. Harris**; *Department of Toxicology, Karolinska Institute, S-10401 Stockholm, Sweden, **Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD 20205

The biological effects of TSC and the smoke-related aldehydes: acrolein, formaldehyde and acetaldehyde, are presently being investigated in cultured human bronchial epithelial cells. Total condensate, a semivolatile fraction and a nonvolatile fraction are each markedly cytotoxic and decrease colony survival and clonal growth at 10 to 100 μg/ml concentrations. Acrolein is more cytotoxic [50% inhibition of CFE (CFE_{50}) occurred at 2 μM] than formaldehyde (CFE_{50}, 400 μM) which in turn is more cytotoxic than acetaldehyde (CFE_{50}, 10 mM). TSC and different fractions obtained by destillation or solvent extractions variably induce squamous differentiation of epithelial cells as judged by increased cell surface area, plasminogen activator activity and formation of cross-linked envelopes. Coincubation with N-acetylcysteine significantly inhibits TSC-induced cytotoxicity and differentiation. Pathobiological effects caused by aldehydes in bronchial cells include reproductive sterilization, enhanced formation of cross-linked envelopes, DNA single strand breaks, DNA protein cross-links, thiol depletion, inhibition of DNA repair and mutations. These studies indicate that several aldehydes and nonvolatile, mainly neutral-methanol extractable, compounds in tobacco smoke cause many pathobiological effects that relate to multistage carcinogenesis in the human bronchial epithelium.

1241 ANALYSIS OF AFLATOXINS IN URINE USING MONOCLONAL ANTIBODY AFFINITY CHROMATOGRAPHY, John D. Groopman, Paul R. Donahue and Gerald N. Wogan, Boston University School of Public Health, Boston MA 02118 and Massachusetts Institute of Technology, Cambridge MA 02139

Monoclonal antibodies specific for aflatoxins were obtained following fusion of mouse SP-2 myeloma cells with spleen cells of mice immunized with aflatoxin B_1 covalently bound to bovine gamma globulin. One of the monoclonal antibodies isolated (2B11) was found to be a high affinity IgM antibody with an affinity constant for aflatoxin B_1, aflatoxin B_2 and aflatoxin M_1 of about 1 x 109 liters per mole. The antibody also has significant cross-reactivity for the major aflatoxin-DNA adducts. The antibody was covalently bound to Sepharose-4B and employed in a column-based solid phase immunosorbent assay system. Aflatoxins added in vitro to samples at levels expected to be obtained in human samples acquired from environmentally exposed individuals were quantitatively recovered from the reusable antibody affinity column. In studies using urine samples from rats injected with radiolabeled [14C]-aflatoxin B_1, we have evidence that aflatoxin metabolites, including aflatoxin M_1, can be isolated by these methods. We have also applied these methodologies to the analysis of human urine samples obtained from people exposed to aflatoxin B_1. Preliminary data, confirmed by HPLC, reveals that aflatoxin M_1 can be quantified in these samples.

1242 DIFFERENTIAL CAPACITIES FOR DNA REPAIR IN VARIOUS CELLTYPES OF THE LUNG Aage Haugen, Toralf Deilhaug, Tore Aune, Hans Krokan and Bjørnar Myrnes. Department of Toxicology, National Institute of Public Health, 0462 Oslo 4, Norway, and Institute of Medical Biology, University of Tromsø, 9001 Tromsø, Norway.

DNA repair plays an important role in protecting the genome against carcinogens. Some chemically induced cancers preferentially appear within organs or subpopulations of cells having low capacity for repair of critical DNA lesions. Respiratory tract tissue is target organ for inhaled xenobiotics known to interact with DNA. The ability to repair damaged DNA was determined in different cell populations of rabbit lung cells isolated by centrifugal elutriation. A dose dependent level of DNA excision repair was observed in alveolar type II cells after exposure to the direct acting alkylating agents N-methyl-N'-nitro-N-nitroso-guanidine (MNNG), N-ethyl-N-nitrosourea (ENU) and methyl methanesulphonate (MMS). Furthermore, O^6-alkylguanine-DNA alkyltransferase activity was easily detectable in alveolar type II cells and alveolar macrophages. In contrast, nonciliated (Clara) cells had 4-20 fold lower levels of DNA excision repair and non-detectable levels of O^6-alkylguanine-DNA alkyltransferase. Uracil-DNA glycosylase activities in Clara cells and alveolar type II cells were in the same range and had 3-fold lower activity than alveolar macrophages. Our findings indicate that various lung cells differ in DNA repair capacity and may thus differ in sensitivity to some carcinogens.

1243 MAMMALIAN ENDONUCLEASE INCISES DNA AT SITES OF PYRIMIDINE OXIDATIVE DAMAGE, Dag E.Helland,Paul W.Doetsch and William A.Haseltine,Dana Farber Cancer Institute, Boston,MA 02150.

We have studied the activity of an endonuclease isolated from calf thymus that in the absence of magnesium incises DNA irradiated with high doses of UV light and DNA damaged by OsO_4, H_2O_2, $KMnO_4$ and γ-rays. End labelled DNA fragments of defined sequences were utilized as substrates.
Following UV-irradiation, enzyme incision of DNA occured exclusively at sites of cytosine wheras with OsO_4 incision occured exclusively at sites of thymine. DNA treated with H_2O_2, $KMnO_4$ or γ-rays was incised by the enzyme at sites of both cytosine and thymine.
Our results suggests that:

1) After UV-irradiation cytosine is converted more frequently than thymine to adducts other than cyclobutane dimers and (6-4)photoproducts.
2) The formation of thymine glycol following UV-irradiation with high doses is minimal.
3) A mammalian enzyme that may mediate an important role in removal of oxidative DNA damage has been found.

The mechanism of DNA incision by this enzyme will be discussed.

1244 REDUCTION OF NEOPLASTIC TRANSFORMATION AT REDUCED DOSE RATES OF ^{60}Co γ-RAYS IS AMPLIFIED BY TPA, C. K. Hill, A. Han and M. M. Elkind. Argonne National Laboratory, Argonne, IL 60439

We have studied transformation in mouse embryo C3H 10T1/2 cells continuously irradiated, at 10 rads/day, throughout the useful life-span of these cells in culture. At cumulative total doses of 50, 150, 300, and 450 rads, aliquots of the cells were assayed for cell survival and neoplastic transformation with or without 0.1 μg/ml of TPA added 24 h after irradiation. Preliminary results suggest that at 10 rads/day neoplastic transformation is reduced to a low level, which is, however, still significantly above background. These results are consistent with previous data recorded at 144 rads/day which showed that early radiation events could be repaired significantly to reduce the frequency of transformation from that observed after single, brief doses [A. Han et al., Cancer Res. 40, 3328, (1980)]. The addition of TPA significantly increases the frequency due to 10 rads/day. Thus far the data suggest that TPA increases the frequency only to the level one would expect from the same dose of irradiation given as a single acute exposure and not to the level seen if the same acute dose of radiation is followed by TPA. These results suggest therefore that during a very prolonged irradiation a slow repair process occurs in addition to the early repair that depends on dose rate. It is clear, however, from the fact that TPA still amplifies transformation that some subeffective damage still remains in the cells even when irradiation is given at the low dose rate of 10 rads/day. (Work supported by the U.S. DOE under contract No. 31-109-ENG-38 and NCI grant No. CA 29940.)

1245 REGULATION OF EXPRESSION OF RAT AND HUMAN CYTOCHROME P450c, R.N. Hines, W.H. Houser, P.L. Iversen, R. Foldes, W. Heiger, R.L. Conrad and E. Bresnick, Eppley Inst. for Research in Cancer, Univ. of Nebraska Med. Ctr., Omaha, NE 68105

The cytochrome P450-dependent monooxygenases (P450) are an inducible family of isozymes involved in the biotransformation of many substances to intermediates capable of interacting with macromolecules, e.g. DNA. To elucidate the mechanism of polycyclic hydrocarbon (PH) induction of P450c, two rat genomic libraries were screened with a P450c cDNA probe and two overlapping clones were isolated. DNA sequence analysis revealed a gene 6.1 kb in length with 7 exons and 6 introns. Potential regulatory sequences both 5' to the gene as well as in intron 1 are being studied. In order to affirm our hypothesis that P450c gene expression is enhanced by interaction at the chromatin level, the ability of the "charged" 4S PH binding protein to interact specifically with total rat DNA or our genomic subclones was examined. Specific binding occurs with radiolabeled total rat DNA and can be effectively inhibited with our unlabeled genomic subclones. We have also examined the steady-state levels of nuclear intermediates to the mature mRNA at different times after a single injection of PH. Our data suggest that nuclear processing is not a rate limiting step in induction. Since the rat gene probes cross-hybridize with human DNA, we have also isolated the human P450c gene. Structural information is highly conserved between the human and rat, but differences in the overall exon/intron structure are seen. Studies are currently in progress to examine the expression of human P450c in lymphocytes and fibroblasts from smokers, non-smokers and cancer patients. Supported by NIH Grant No. CA36106 and Nebr. State Dept. Hlth. Grant No. 85-25.

1246 MOLECULAR AND CELL BIOLOGICAL CHARACTERIZATION OF A HUMAN GENE INVOLVED IN EXCISION REPAIR, Jan H.J. Hoeijmakers, Andries Westerveld, Marcel van Duin, Jan de Wit, Hannie Odijk, Marcel Koken and Dirk Bootsma.

The autosomal recessive syndrome xeroderma pigmentosum (XP) is characterized by extreme sensitivity of the skin to sunlight and predisposition to skin cancer. The primary defect in most XP patients is thought to be a deficiency in the excision repair of DNA lesions. The mechanism of this main DNA repair pathway in mammalian cells is poorly understood. To investigate the genes and proteins involved in excision DNA repair we have recently cloned a human gene complementing the excision repair defect of a UV and mitomycin C (MM-C) sensitive Chinese hamster cell line (CHO43-3B) (Westerveld et al., Nature 210, 425, 1984). The cloning of this gene, designated ERCC-1 was archieved by gene transfection and cosmid cloning in E.coli. After transfection ERCC-1 induces repair proficiency in CHO43-3B cells with respect to UV and MM-C survival, dimer removal and UDS. It has a size of 15-16 kbp and contains at least 8 exons. One major RNA (1.1 kb) hybridizing with ERCC-1 probes is found in a variety of human cells. Preliminary experiments suggest that UV-irradiation has no major effect on their transcription. Nucleotide sequence information of the 1.1 kb RNA and data on the chromosomal localization of ERCC-1 will be presented. ERCC-1 is not deleted or grossly rearranged in cell lines from XP complementation groups A,C,F and G, and does not induce repair proficiency after transfection to SV40 transformed XP-A and F fibroblasts. The relationship of ERCC-1 with other XP complementation groups and other human syndromes associated with DNA repair defects such as Fanconi's anemia, is presently under investigation.

1247 PROPERTIES OF A MAMMALIAN 3-METHYLADENINE DNA GLYCOSYLASE, Kjell Kleppe, Rune Male, Dag Helland, Bjørn Ivar Haukanes and Karl-Henning Kalland, Department of Biochemistry, University of Bergen, Bergen, Norway.

3-Methyladenine-DNA glycosylase from calf thymus has been purified and characterized. Two species of molecular weight 42 and 27 kd were found. In the nucleus only the low-molecular weight form was present bound to the chromatin. The enzymatic properties of the two species appeared to be similar. They both released 3-methyladenine, 7-methylguanine and 3-methylguanine, listed in order of decreasing activity. The chromatin associated enzyme was purified to apparent homogeneity and found to be a basic protein having a pI greater than 9. It was inhibited by p-hydroxy mercuribenzoate, but this inhibition could be completely reversed by addition of excess 2-mercaptoethanol. A number of different experiments demonstrated that the 3-methyladenine and 7-methylguanine releasing activities were located on the same molecule. The K_m for the release of 3-methyladenine was found to be approximately $5x10^{-8}$ M using calf thymus DNA as a substrate and $5x10^{-9}$M with $d(A-T)_n$. The corresponding K_m for 7-methylguanine release was $5x10^{-9}$ both for calf thymus DNA and $d(G-C)_n$. Addition of 0.1 mM spermine gave a marked increase in the rate of release of bases using synthetic DNA substrates, whereas the K_m increased approximately 10 fold. The mechanism of action of the enzyme has also been investigated with DNAs of defined sequences.

1248 RECOMBINANT DNA EXPRESSION VECTORS FOR STUDY OF DNA REPAIR IN HUMAN CELLS. M. Protić-Sabljić and K.H. Kraemer, Laboratory of Molecular Carcinogenesis, NCI,Bethesda, MD.

We have developed a novel host-cell reactivation assay of DNA repair utilizing UV-irradiated plasmid vectors. We measured transient expression of transfected genes 48 hours after transfer into human cells. This assay primarily reflects transcriptional activity of the transfected DNA. UV-irradiated plasmids pSV2catSVgpt (7.3 kb), pSV2cat (5 kb), and pRSVcat (5.2 kb) were used. Chloramphenicol acetyltransferase (CAT) and xanthine phosphoribosyltransferase (XPRT) activities were measured by TLC. UV-induced dimer formation in the plasmids was quantitated by the disappearance of form I DNA on agarose gels following irradiation and T4-endonuclease V treatment. In comparison to normal cells, xeroderma pigmentosum (XP) cells (complementation groups A and D) expressed UV-damaged genes at much lower levels than they do non-irradiated genes. The D_{37} of the CAT inactivation curve was 58 J/m^2 for pSV2catSVgpt, 48 J/m^2 for pSV2cat and 51 J/m^2 for pRSVcat in XP12BE(SV40) cells and about 800 J/m^2 in normal cells (GM0637(SV40)). The similarity of the D_{37} data in these XP group A cells for 3 plasmids of different size suggest they all have the same UV-inactivation target size. Thus one UV-induced dimer inactivates a target of about 2 kb, the size of the cat expression unit. The D_{37} of the XPRT inactivation curve was about 136 J/m^2 in XP A cells. This implies a smaller target (0.7 kb) than the gpt expression unit (2.2 kb). Experiments with acid-heat induction of O-4 AP sites in pSV2catSVgpt indicate the same inactivation of cat in XP groups A and D as in normal cells. This study demonstrates that XP cells are defective in transcription of UV-damaged transfecting DNA. The transcriptional activity of damaged recombinant DNA expression vectors may be useful for assessing repair of DNA in human cells.

1249 INITIAL CHARACTERIZATION OF THE UNIQUE ARYLAMINE/DNA ADDUCT FORMED BY ACTIVATION OF 2-AMINOFLUORENE BY PROSTAGLANDIN H SYNTHASE. Robert S. Krauss and Thomas E. Eling NIEHS/NIH Research Triangle Park, NC 27709

We have previously demonstrated that peroxidative activation of 2-aminoflourene (2-AF) by prostaglandin H synthase in the presence of calf thymus DNA *in vitro* leads to the formation of DNA adducts that are polar (i.e., not adsorbing to Sephadex LH-20, poor extraction into 1-butanol) and reactive (i.e., binding to glassware, adsorbing to DNA digestive enzymes) (Krauss and Eling, Cancer Res., in press,1985). Using a technique that employs extraction of the adducts with 0.1 M tetrabutylammonium chloride/1-butanol, porous-resin preparative HPLC, and reversed-phase HPLC, we have observed that one major adduct is formed in this system. The purified adduct shows UV absorbance maxima at 327 and 270 nm, and does not absorb past 360 nm. pH-dependent partitioning of the adduct between aqueous and organic solvents indicates that the adduct possesses two acidic ionizable groups, one with pK_a between 3 and 4, the other with pK_a between 10 and 11. Thus, the adduct appears to be negatively charged at neutral pH. This provides an explanation for both the polarity and reactivity of this adduct. Moreover, the spectral and solvent partitioning data suggest the adduct is not a quinoneimine-type adduct with extended conjugation between the carcinogen and DNA base. Unequivocal identification of this unique adduct by mass spectrometry and ^1H-NMR spectroscopy is underway. Upon structural identification of the adduct, we hope to use it as a biochemical endpoint with which to investigate the role of peroxidative activation of 2-AF in canine urothelium *in vivo*.

1250 ALTERED AP-ENDONUCLEASE IN AN AT CELL LINE: IMPLICATION OF A SECONDARY PROTEIN MODIFICATION, Urs Kuhnlein, Chalk River Nuclear Laboratories, Chalk River, Ontario

We have compared apurinic/apyrimidinic (AP) endonuclease from HeLa cells and the SV40-transformed dermal cell line AT5BIVA from a patient with the repair-deficiency syndrome ataxia telangiectasia (AT). The two enzymes differ in their molecular weight (HeLa: 37,600; AT: 38,900) and their dissociation equilibrium constant for AP-sites (HeLa: 7.8 10^{-11} M; AT: 28.3 10^{-11} M). These variances might be the consequence of a different post-translational modification of the two enzymes. Evidence for this interpretation stems from the observation that the AP-DNA binding activity of AP-endonuclease, as measured in a glass-fiber filter binding assay, is inactivated upon incubation of the enzyme with partially purified snake venom phosphodiesterase and that the AP-endonuclease from AT cells is 5-fold more sensitive than the HeLa enzyme. For both enzymes the diesterase treatment leads to the formation of a protein of MW 35,500 which might be the unmodified precursor protein of AP-endonuclease. The loss of AP-DNA binding activity does not reduce but rather increases the catalytic activity of AP-endonuclease when measured at excess substrate concentration. Experiments are now in progress to determine the molecular nature of this putative post-translational modification and to establish whether an altered AP-endonuclease is characteristic of the AT genotype.

[Supported by the NCI of Canada, the MRC of Canada (MA-6782) and by the US NCI Contract NO1-CP-21029 (Basic). The author is also affiliated with the Department of Medical Genetics, University of British Columbia, Vancouver, BC.]

1251 IN VITRO ENZYMATIC METHYLATION OF BROMODEOXYURIDINE DENSITY LABELLED 'DNA. Mathuros Ruchirawat and Jean-Numa Lapeyre, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030

Bromodeoxyuridine (BrdU) density-labelled parental and filial strands of phage M13 DNA were used to study the mechanism of maintenance DNA methylation on hemimethylated DNA and de novo or repair type methylation on double- and single-stranded DNA. 1500-fold purified rat liver DNA methyltransferase catalyzed de novo methylation on single- and double-stranded DNA was found to obey Michaelis-Menten (M-M) kinetics and be non-processive; however, maintenance methylation of hemimethylated DNA did not obey M-M kinteics but showed highly cooperative kinetics with respect to the concentration of methylatable sites. Rapid reequilibration of the enzyme from double- and single-stranded DNA could be demonstrated by challenge with hemimethylated competitor DNAs whereas the converse could not be demonstrated suggesting facilitated transfer instead of a 3-dimensional diffusion mechanism for enzyme translocation on hemimethylated, newly replicated DNA. BrdU-substitution had no effect on the K_m of methylation reactions but slowed down the velocity approximately 50%. This effect may be of significance for experiments employing BrdU to density label DNA in order to study rates of enzymatic methylation in carcinogen- or UV-induced repaired DNA tracts.

1252 PRODUCTION OF ANTIBODIES AGAINST T4 ENDONUCLEASE V AND EXPRESSION OF THE denV GENE IN PHAGE λgt11, R. Stephen Lloyd, Vanderbilt University, Nashville, TN 37232.

The pyrimidine dimer specific endonuclease from the bacteriophage T4 was purified to apparent homogeneity and was used to raise polyclonal antibodies in rabbits. By Western analyses these antibodies recognize a single 16.5 K dalton protein from E. coli extracts infected with wild type T4. These antibodies have also been shown to completely inhibit the pyrimidine dimer specific nicking activity of the endonuclease V as measured by the lack of conversion of form I UV irradiated plasmid DNA into nicked circular DNA (form II) by the enzyme. In addition a library of EcoRI cut T4 DNA was made in phage λgt11. Portions of this expression phage library (350,000 members) were plated on E. coli Y1090 and induced at 42°C with 2 mM IPTG and plaques were screened for the production of immunologically reactive endonuclease V protein. Numerous immunologically reactive plaques were found and all contain at least a 1.8 kb EcoRI T4 fragment. By Western analyses all phage which were selected as immunologically positive, produce a fusion protein with β-galactosidase.

1253 CELLS FROM XERODERMA PIGMENTOSUM (XP) VARIANTS ARE ABNORMALLY SENSITIVE TO TRANSFORMATION BY UV, BUT THEIR EXCISION REPAIR PROCESS IS NORMAL IN RATE AND "ERROR-FREE," V. M. Maher, S. K. Kohler, M. Watanabe and J. J. McCormick, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824.
Although XP variant patients have all the clinical characteristics of the XP disease (genetic predisposition for sunlight-induced cancer), their cells have a normal rate of excision of UV-induced DNA damage. We showed previously that fibroblasts from such patients are abnormally sensitive to mutations induced by UV or by simulated sunlight. We compared these cells to normal cells for the frequency of transformation to anchorage independence induced by UV. The XP variant cells exhibited a high frequency (5×10^{-4}) at doses for which the normal cells showed no induction. Since this sensitivity is not caused by lack of excision repair (as is the case for classical XP's), one possible explanation is that XP variant cells introduce mutations during excision ("error-prone"). We tested this by comparing normal and XP4BE cells for the frequency of mutations induced in synchronized populations irradiated at the beginning of S-phase, or in early G_1 (12 to 18 hr prior to onset of DNA synthesis), or in the confluent G_0 state under conditions designed to permit them 24 hr for excision repair prior to onset of S. Although, as expected, the decrease in mutant frequency with time for excision before S was the same for both kinds of cells, i.e., 66% lost in 12 hr; 80% lost in 24 hr, under each condition the XP4BE cells exhibited a higher frequency of mutants than did the normal cells. In fact, the frequency induced in the XP4BE cells irradiated at the onset of S approximated that induced in excision-minus XP cells. Supported by NIH NCI grants CA21289 and CA21253 and by DOE Contract 0459.

1254 PULMONARY ACTIVATION OF CARCINOGENS BY BABOONS, Milton V. Marshall and Walter R. Rogers, Southwest Foundation for Biomedical Research, San Antonio, TX 78284
Cigarette smoke is a complex mixture of carcinogens, cocarcinogens, and anticarcinogens. Through metabolic activation, precarcinogens such as polycyclic aromatic hydrocarbons (PAHs) are converted to ultimate carcinogenic (and mutagenic) species. This association between metabolic activation and cigarette smoking as related to lung cancer is under investigation in a group of cigarette-smoking baboons. These nonhuman primates have been taught to smoke in a human-like manner through operant conditioning; the 20 animals in this study have a cigarette smoke exposure varying between 1 and 15.5 pack-years (mean = 9.9 for chronic smokers). Pulmonary alveolar macrophages (PAMs) were obtained by lavage and bronchial epithelial cells were obtained by biopsy. Chronic smokers and sham-puffing controls were initially compared for the ability to activate benzo(a)pyrene (BaP). The sham-puffing baboons were then introduced to cigarette smoking and these novice smokers were compared with the chronic smoking group. No differences between groups were observed in the metabolism of BaP. When a cell-mediated assay was employed with V79 cells as target cells, PAMs from cigarette smokers activated the BaP-7,8-dihydrodiol to a greater extent than the sham-puffing group. After 4 months of smoking, PAMs from the novice smokers activated BaP-7,8-dihydrodiol to a significantly greater extent than before smoking. Although this activity was lower than that of chronic smokers, it increased to similar levels after an additional 5-month exposure. Other respiratory carcinogens (PAHs and nitrosamines) were activated in the PAM-mediated V79 mutagenicity assay. Supported by PHS grant 1 R01 CA33069 awarded by the NCI.

1255 CORRELATION BETWEEN 0^6-ALKYLGUANINE-DNA ALKYLTRANSFERASE (MT) ACTIVITY AND RESISTANCE OF HUMAN CELLS TO THE CYTOTOXIC AND MUTAGENIC EFFECTS OF METHYLATING AND ETHYLATING AGENTS, J. J. McCormick, J. Domoradzki, R. C. Corner and V. M. Maher, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824.
Cells from Gardner's syndrome (GS) and familial polyposis coli (FP) patients, were compared to those of normal persons for sensitivity to the cytotoxic and mutagenic action of MNNG and ENU. FP cell line GM2355 and GS cell lines 2938 and GM3948 exhibited normal sensitivity to the cytotoxic and mutagenic effects of these agents. In contrast, GS cell line GM3314 and cells from an apparently normal fetus (GM0011) were abnormally sensitive to both agents. To determine if the resistance of the various cell lines correlated with their ability to remove methyl or ethyl groups from the 0^6-position of guanine, we measured the MT activity. The normally resistant lines exhibited normal levels of MT; the sensitive strains showed virtually non-detectable levels of this activity. We also compared fibroblasts from a XP patient (XP12BE, complementation group A), an SV40 virus-transformed XP cell line (XP12ROSV) and a normal cell line transformed by this virus (GM637) for their response to these 2 agents and for their level of MT. XP12BE cells had a normal level of MT activity; GM637 had a reduced level; and XP12ROSV showed no activity. XP12BE cells showed a normal response to MNNG; GM637 gave an intermediate response; XP12ROSV cells were very sensitive. When treated with ENU, GM637 cells gave an intermediate response, but both XP12BE and XP12ROSV were sensitive. These results suggest that 0^6-alkylguanine and/or any other adduct repaired by MT is a potentially cytotoxic and mutagenic lesion. They also suggest that nucleotide excision repair, which is deficient in XP cells, plays a role in repair of the potentially lethal and mutagenic damage induced by ENU. Supported by CA21253.

1256 O^6-METHYLGUANINE REPAIR IN HUMAN FIBROBLASTS, M. Middlestadt, R. McWilliams, R. Mirzayans, G. Norton and M. Paterson, Chalk River Nuclear Labs., Chalk River, Ont.

In ongoing studies of genetic-environmental interactions in cancer causation we have identified a number of nontransformed dermal fibroblast strains, from persons with or prone to cancer, which display an abnormal sensitivity towards killing by N-methyl-N-nitrosourea (MNU). These include strains from (i) three female members of a family with Gardner syndrome, in whom the characteristic predisposition to colon cancer is manifested; (ii) a patient with AIDS; and (iii) an individual who received chemotherapy and subsequently developed additional neoplasms. Moreover, a fetal fibroblast strain, hypermutable by MNU, exhibits an *in vitro* age-dependent increase in MNU-induced cytotoxicity. In all instances the observed cellular chemosensitivity is associated with defective O^6-methylguanine (O^6-MeG) repair. While normal fibroblasts possess an average O^6-MeG-DNA methyltransferase (MT) activity of approximately 10^5 molecules/cell, these MNU-sensitive strains, in comparison to appropriate age-matched controls, contain 0-50% of this constitutive level; the relative MT deficiency parallels the relative degree of MNU hypersensitivity. Follow-up studies are presently underway to determine (i) if other factors, in addition to reduced basal levels of MT activity, contribute to the observed chemosensitivity of these strains; and (ii) if there is complementation among the mutant strains with respect to MT expression.

[Supported in part by US NCI Contract NO1-CP-21029 (Basic)]

1257 DETECTION OF ALKYLATED BASES AND ALKYLTRANSFERASE ACTIVITY IN HUMAN TISSUES. R. Montesano, D. Umbenhauer & C.P. Wild, Intern. Agency for Res. on Cancer, Lyon, France; R. Saffhill & J.M. Boyle, Paterson Labs, Manchester UK; N. Huh, U. Kirstein & M.F. Rajewsky, Inst. for Cell Biol. Tumour Res., Essen FRG; S.H. Lu, Cancer Inst. Chinese Acad. Med. Sci., Beijing, People's Republic of China.

The availability of highly sensitive immunoassays has allowed us to measure levels of O^6-alkyldeoxyguanosine in human oesophageal and stomach samples as a method of monitoring exposure to environmental alkylating agents. Human surgical samples were obtained from patients in Linxian county, People's Republic of China, an area where food analysis has provided some evidence of dietary exposure to nitroso compounds and where there is a concomitant high risk of development of oesophageal cancer. Quantities of 2-13 mg DNA were isolated from oesophageal tumours, non-invaded oesophagus or cardiac stomach tissues and following enzymic digestion the DNA hydrolysates were fractionated by HPLC, which separates the 4 parental deoxynucleosides to be individually quantitated spectrophotometrically whilst the levels of O^6-MedG and O^6-EtdG may be determined by radioimmunoassay (RIA). The limit of detection in an RIA for O^6-MedG is 20 fmol so that even with the lowest quantity of DNA (2 mg) used in this study a level of 0.02 µmoles O^6-MedG per mol DNA deoxynucleoside (dX) can be determined. In a total of 22 non-tumourous oesophageal samples, 6 had O^6-MedG levels > 0.02 µmoles O^6-MedG per mol dX with the highest level of modification being 0.044 µmoles O^6-MedG per mole dX. Only one of the twelve stomach samples assayed had such a high level of adducts and none of the tumour samples or control DNA samples had levels this high. No O^6-etdG was detected in these samples. In parallel the O^6-alkylguanine DNA transferase activities were determined.

1258 DETECTION OF ANTIBODIES TO BENZO(a)PYRENE DIOL EPOXIDE-DNA ADDUCTS FOLLOWING OCCUPATIONAL EXPOSURE TO BENZO(a)PYRENE, M.J. Newman*, K. Vahakangas, G.E. Trivers, D.L. Mann, W.W. Wright** and C.C. Harris, Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD 20205; *Uniformed Services University of the Health Sciences, Bethesda, MD 20814; **USC School of Medicine, Los Angeles, CA 90033

Benzo(a)pyrene is a common chemical carcinogen found in tobacco smoke, atmospheric pollution and smoked foods. Benzo(a)pyrene occurs in the environment in a procarcinogenic form that is activated metabolically to form the carcinogen, diol-epoxide. The diol-epoxide binds primarily to the exocyclic-amino groups of deoxyguanine to form the adducts with the major adduct being 7β,9α-dihydroxy-9α,10αepoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDE-DNA). Exposure of humans can be particularly high through the polluted atmosphere of certain occupations and/or the smoking of tobacco products. To investigate the effects of exposure on humans, we quantitated the levels of BPDE-DNA and the levels of serum antibodies specific to the same adduct in the blood of 41 coke oven workers some of which were smokers. Detectable levels of BPDE-DNA were observed in the lymphocyte DNA in 2/3 of the coke oven workers with the highest levels being associated with the smoking of tobacco. Specific serum antibodies were detected in about 1/3 of these same individuals. The data suggest that environmental exposure to benzo(a)pyrene can lead to the generation of BPDE-DNA adducts and antibodies against these DNA adducts in some individuals. The possible significance of the serum antibodies and their role in cancer risk is as yet unknown.

1259 SPECIFIC CARCINOGEN MODIFICATION OF DNA FROM EARLY S PHASE HUMAN CELLS LEADING TO THE EXPRESSION OF A TRANSFORMED PHENOTYPE, Martin Ribovich and George Milo, The Ohio State University, Columbus, Ohio 43210

One of the most difficult facets of examining carcinogen modification of DNA in human cells is the availability of numbers of cells and the extent of sensitivity of measurement where a transformed phenotype can be identified. The ^{32}P-postlabeling method was used to identify specific carcinogen-DNA adducts from treated DNA of human cells. Cells ($4.5X10^6$) were treated for 3 hr. in early S phase of the cell cycle with 1.14uM(±)BPDE-I. DNA was isolated, digested, and 170 ng postlabeled. After TLC and autoradiography, six spots were identified that were not present in the control. Corollary experiments with calf thymus DNA-BPDE-I gave us equivalent spots on TLC. Preliminary identification of the adducts was made by cochromatography on TLC with both adducts obtained from reacting BPDE-I with deoxyribonucleoside 3'-monophosphates (3'-PdG, 3'-PdA, 3'-PdC) and adducts from [^3H]-BPDE-I-DNA. In the latter case, the individual adducts (^3H, ^{32}P) were isolated, dephosphorylated, and the [^3H]-nucleosides cochromatographed on HPLC with known standards. Major adducts from treated human cells were identified as the 3',5'-diphosphate of the 7R and 7S anti-BPDE:trans-N^2-dG nucleosides. Their levels of modification were 12 and 5 adducts/10^6 nucleotides, respectively. The lowest level of specific modification was 1.6 adducts/10^7 nucleotides. This work establishes that a highly sensitive measurement of specific DNA adducts can be achieved and that these low levels of DNA modification subsequently lead to a fully initiated cell and expression of a transformed phenotype. This work was supported by an award from EPA R810407

1260 EFFECTS OF CHRONIC HEPATOCARCINOGEN EXPOSURE: I. MOLECULAR DOSIMETRY, F.C. Richardson, M.C. Dyroff, M.A. Bedell, J.A. Boucheron, and J.A. Swenberg, Chemical Industry Institute of Toxicology, Research Triangle Park, NC

A series of experiments have been performed to determine the molecular dosimetry of DNA adducts in the target organ and cell. Specifically, these studies have measured the accumulation of the promutagenic adducts O^4-alkylthymidine and O^6-alkylguanine in rat liver and hepatocyte DNA during continuous exposure to either 30 ppm 1,2 dimethylhydrazine (SDMH) or 40 ppm diethylnitrosamine (DEN) ad libitum in drinking water. Following 28 days of DEN exposure, alkylation levels were < 0.2 pmoles O^6-ethylguanine/umole guanine and 10.0 pmoles O^4-ethylthymidine (O^4-Etdt)/umole thymidine (dT), while 28 days of SDMH generated concentrations of 1.6 pmoles O^6-methylguanine/umole, guanine and 0.8 pmoles O^4-methylthymidine/umole thymidine. Differences in the sum of the promutagenic adducts generated by these essentially equimolar doses of DEN and SDMH correlate well with the greater efficacy of DEN vs SDMH as a hepatocarcinogen. Within the liver, results demonstrate that by 14 days of DEN exposure, O^4-ethylthymidine concentrations were higher in the right median (4.7 pmoles O^4-EtdT/umole dT) and left (8.4 pmoles O^4-EtdT/umole dT) versus the right anterior (3.3 pmoles O^4-EtdT/umole dT) hepatic lobe. These interior differences in O^4-EtdT concentrations correlate well with results demonstrating lobe preferences in DEN induced hepatocellular carcinoma.

1261 LYMPHOCYTES AS A MODEL TO STUDY REPAIR OF DNA LESIONS INDUCED IN VIVO, Felix Romagna, Colleen Hunnicutt, Julie Angerman-Stewart and Marshall Anderson, The National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

DNA repair was examined in lymphocytes of rats treated with a single dose of either N-methyl-nitrosourea (MNU) or 4-nitroquinoline-1-oxide (4NQO). After treatment of animals with carcinogens, lymphocytes were isolated, incubated in vitro for 24 hours and DNA repair measured throughout the incubation period by the nucleoid sedimentation assay and unscheduled DNA synthesis (UDS). Lymphocytes had the capacity to repair in vitro DNA damage induced in vivo by MNU and 4NQO. Moreover, the in vitro repair rates corresponded to those measured in vivo. Incubation of lymphocytes obtained from treated animals with the polymerase α inhibitor aphidicolin, resulted in an accumulation of repair-induced DNA strand breaks. Moreover, the ability to accumulate DNA strand breaks in the presence of the inhibitor allowed us to detect DNA damage induced by low doses of these carcinogens. The effect of aphidicolin was reversible. After the removal of the inhibitor from the incubation medium, the partially inhibited excision repair process was then completed. Thus, the use of the in vitro lymphocyte system in conjunction with specific inhibitors of DNA repair should be a valuable tool to detect low levels of in vivo carcinogen-induced DNA damage and to study the mechanism of excision repair.

1262 EFFECTS OF PREIRRADIATION OF HOST CELLS UPON REPLICATION OF UV-DAMAGED SIMIAN VIRUS 40, Abraham Scaria and Howard J. Edenberg, Dept. of Biochemistry, Indiana Univ. School of Medicine, Indianapolis, Indiana 46223.

Irradiation of E.coli induces expression of genes involved in DNA repair and mutagenesis ("SOS response"). Indirect evidence has suggested an inducible response in mammalian cells also; preirradiation of host cell increases the mutation frequency of UV irradiated Simian virus 40 (SV40). We are examining the effects of preirradiation of the host cells upon the replication of UV-damaged SV40. Control cells and cells preirradiated with low fluences of ultraviolet light were infected with SV40, and the effects of a subsequent irradiation were determined. SV40 replication was assayed as incorporation of tritiated dT into viral DNA, normalized to the prelabeled viral DNA present in the cells. The inhibition of SV40 replication by UV was less pronounced in preirradiated cells. Density labeling showed that preirradiation of the host cells increased the fraction of newly replicated viral DNA molecules that reentered and completed replication after UV-damage. A higher percentage of the SV40 molecules from preirradiated cells were covalently closed molecules. These results suggest that preirradiation of the host cells mitigates the effect of a subsequent UV exposure.

1263 ANTIPAIN-MEDIATED SUPPRESSION OF SISTER CHROMATID EXCHANGES INDUCED BY INHIBITORS OF POLY (ADP-RIBOSE) POLYMERASE, Jeffrey L. Schwartz and Ralph R. Weichselbaum, University of Chicago, Chicago, IL 60637.

Exposure of mammalian cells to inhibitors of poly (ADP-ribose) polymerase such as 3-aminobenzamide (3AB) results in the induction of sister chromatid exchanges (SCEs). The mechanism for the induction by 3AB is unknown, but there are some similarities between SCE induction by 3AB and the high SCE frequency found in Bloom's Syndrome cells. Certain protease inhibitors such as antipain have been reported to inhibit SCE induction in Bloom's Syndrome cells. We have found that antipain will also suppress 3AB-mediated SCE frequency. As has been found with Bloom's Syndrome cells, the effects are partial and are saturated at 30 μm concentrations of antipain. Antipain has no effect on baseline or alkylation-induced SCEs but will reduce SCE frequency induced by high exogenous levels of nucleotides in a similar fashion as those induced by 3AB. These effects appear to involve free radical production because dimethyl sulfoxide a free radical scavenger will mimic antipain effects.

1264 DOES CARCINOGENIC TREATMENT LEAD TO INDUCTION OF UNTARGETED MUTAGENESIS IN MAMMALIAN DNA? J.W.I.M. Simons(1) and A.G.A.C. Knaap(2), (1)Department of Radiation Genetics and Chemical Mutagenesis, Leiden, The Netherlands, (2)National Institute of Publick Health, Bilthoven, The Netherlands

Treatment of mammalian cells with carcinogens leads to a transient enhancement of the spontaneous mutation frequency in untreated virus that is growing in these cells. This phenomenon has been described as untargeted mutagenesis. Recently it has been indicated that this process is altered in some syndromes which are characterized by enhanced tumor formation. It is still unknown whether this untargeted mutagenesis also takes place in the mammalian DNA itself. Therefore a method has been developed to measure the spontaneous mutation frequency directly in mutagenized mammalian cells. The procedure consists of splitting a mutagenized cell population in subcultures with low numbers of cells so that cultures with induced mutants can be distinguished from cultures with spontaneous mutants on the basis of their mutant frequencies. Subsequently the spontaneous mutation frequency can be calculated by means of the fluctuation test for the cultures without induced mutants. The results so far obtained with UV-irradiation, X-irradiation and ethyl-nitrosourea do not indicate an enhancement of the spontaneous mutation frequency in mutagenized mouse lymphoma cells.

1265 SCANNING ELECTRON MICROSCOPIC ANALYSIS OF CHROMOSOME DAMAGE, Patricia P. Smith, Diane M. Durnam, Joan C. Menninger and James K. McDougall, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

We are using scanning electron microscopy (SEM) to explore the mechanisms of chromosome damage and repair. Specific chromosome breakage and rearrangements have been associated with neoplasias as well as birth defects and genetic diseases. We are taking two approaches, first, the examination of human cells infected with the highly oncogenic adenovirus Type 12 (Ad 12). Ad 12 causes specific breaks in human chromosomes 1 and 17, which are also frequently involved in chromosome aberrations in human neoplasias. Preliminary results from SEM have allowed identification of the specific virus-induced "uncoiler" regions within which a number of gene loci have been mapped. We hope to elucidate the nature of the remaining chromatin, i.e. altered chromatin structure, DNA uncoiling or other ultrastructural changes.

Our second approach is to examine the ultrastructure of chromosomes which have translocated. We are examining translocated chromosomes from leukemic patients at the Hutchinson Center as well as characterized neoplastic cell lines. The translocated regions are being examined to explore possible chromatin alteration in the reassociated areas.

1266 THE RAT VENTRAL PROSTATE: INTERACTIONS OF A MAJOR SECRETORY PROTEIN AND THE RECEPTOR FOR 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN IN THE REGULATION OF CYTOCHROME P-450. Peter Söderkvist,Lorenz Poellinger and Jan-Åke Gustafsson, Dept. Medical Nutrition,Karolinska Institute, Huddinge University Hospital, Huddinge, Sweden.

Epidemiologic studies have provided evidence that a major fraction of human cancer is due to environmental factors. In line with this contention a chemical etiology of prostatic cancer is suggested. We have adressed the issue if the rat ventral prostate possesses enzymes necessary for the metabolic activation of chemical carcinogens and found that polycyclic aromatic hydrocarbons (PAH:s) such as B-naphthoflavone and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are potent inducers of certain isozymes of cytochrome P-450 and benzo(a)pyrene (BP)metabolism. Furthermore, the contention was tried if this induction process was mediated by a soluble receptor mechanism described for the regulation of cytochrome P-450 genes in hepatic tissues, a mechanism strikingly similar to the current concept of gene regulation by steroidhormones. Using high resolution gel permeation chromatography ^{3}H-TCDD labeled prostatic cytosol contain 5-20 fmol TCDD-receptor/mg protein. High levels of non-specific binding to a major steroid-binding protein (PSP) were observed during receptoranalysis. The affinity of ^{3}H-TCDD for purified PSP is 10-100 times lower than for TCDD-receptorbinding sites, with an apparent K_d of 2-20 nM. It is speculated whether this high capacity binding protein is of any importance in the receptor-mediated induction process, in tissue specific accumulation of chemical carcinogens and in the ensuing metabolic activation of such compounds.

1267 ENHANCED TRANSFORMATION OF HUMAN CELLS BY pSV2 PLASMIDS CONTAINING "BULKY" LESIONS, Graciela Spivak, Ann K. Ganesan, Steven A. Leadon, Jean Michel Vos, Stephanie Meade and Philip C. Hanawalt, Stanford University, Stanford, CA 94305.

We observe an enhancement in the yield of Gpt+ transformants when the chimeric plasmid, pSV2-gpt is irradiated with UV (254 nm) prior to being introduced into human cells by calcium phosphate coprecipitation. UV doses up to 200 J/m^2 cause dose dependent increases in transformation frequency while doses above 1 kJ/m^2 are required to reduce the frequency of transformation below that of unirradiated DNA (Spivak et al., 1984, Mol. Cell. Biol. 4:1169-1171). Enzymatic photoreactivation of irradiated plasmid DNA reduces the frequency of transformation to nearly that of unirradiated DNA, indicating that pyrimidine dimers contribute substantially to enhancement. Other "bulky" lesions, e.g. hydroxymethyltrimethylpsoralen monoadducts and crosslinks, also enhance transformation frequencies, while "non bulky" lesions, e.g. thymine glycols and apurinic sites, do not affect the efficiency of transformation. To study the effect of lesions inside and outside of the gpt gene, respectively, pSV2-gpt was digested with Pvu II and Bam Hl. The 2.3 kb fragment containing the gpt gene and most of the SV40 sequences, and the 3 kb fragment containing the pBR322 sequences were separated. The plasmid was reconstituted from irradiated and unirradiated fragments in various combinations and used for transformation. UV irradiation of the gpt-containing fragment was deleterious to transformation, while irradiation of the pBR322-containing fragment enhanced transformation. Our current hypothesis is that certain lesions in plasmid DNA promote its integration into the recipient genome while the same lesions in the gpt gene block its expression. Supported by grants from the American Cancer Society and the USPHS.

1268 PERSISTENCE OF ALKYLATION - INDUCED DAMAGE IN HETEROCHROMATIC DNA. Bernard W. Stewart, Ewan J. Ward and Michelle Haber, Childrens Leukaemia and Cancer Research Unit, Prince of Wales Childrens Hospital, Randwick, N.S.W. 2031 and School of Pathology, University of New South Wales, Kensington, N.S.W. 2033, AUSTRALIA.
We have analyzed rat liver DNA extracted from whole tissue as well as euchromatic and heterochromatic fractions generated from isolated nuclei by mild micrococcal nuclease digestion. Comparison of structural defects in DNA was made before and after treatment of animals with a non-necrotizing dose of the alkylating carcinogen dimethylnitrosamine (DMN). DNA was radioactively labelled by incorporation of tritiated thymidine,either administered before or after the carcinogen to label parental or daughter strands respectively. Structural defects in DNA were studied in mechanically sheared preparations using analytical benzoylated DEAE (BD)-cellulose chromatography. In this manner single stranded regions ('gaps') of 10 to 20,000 bases in otherwise double stranded DNA can be compared. Over a 10 day period following carcinogen treatment, structural changes consistent with DNA repair activity persisted in heterochromatic DNA. The data suggest a differential turnover of DNA between the two chromatin fractions implying preferential repair of transcriptionally active DNA. Persistent repair activity in heterochromatic DNA appears to block replication induced by hepatectomy between 4 and 24 hours following DMN injection. When replication does proceed, structural change in heterochromatic DNA persists.

1269 EFFECTS OF CHRONIC HEPATOCARCINOGEN EXPOSURE: II. GGT+ FOCI INDUCTION. J.A. Swenberg, F.C. Richardson, M.C. Dyroff, J.A. Popp, and P.H. Morgan, Chemical Industry Institute of Toxicology, RTP, NC

Initiation is considered an irreversible event in the multistage process of carcinogenesis. If carcinogen induced promutagenic adducts and concurrent cell replication result in initiation, the number of initiated cells should increase with continued exposure carcinogen exposure or higher rates of cell replication. We have investigated this possibility in livers of rats continuously exposed to DEN and SDMH, using GGT+ foci as a phenotypic marker of initiated hepatocytes. Utilizing a modified Solt-Farber growth selection regimen, a 4 week exposure to DEN beginning at 4, 8, 10, 12 or 14 weeks of age resulted in 522, 76, 48, 54, 34 GGT+ foci/cm^3, respectively. This difference disappeared when foci were normalized to body weight gain, suggesting that cell replication was a major determinant in the number of GGT+ foci induced. Using 4 week old animals and a modified Cayama-Farber growth selection regimen to measure GGT+ foci induction in several hepatic lobes, it was found that the left and median lobes had approximately 2 times as many foci as the right anterior lobe. These data correlate well with the demonstrated lobe preference for both O^4-ethylthymidine accumulation and induction of hepatocellular carcinoma. Chronic administration of SDMH or DEN resulted in an increased and subsequent plateauing of GGT+ foci with increasing time of exposure. However the number of SDMH induced foci peaked at ~130 foci/cm^3, while the number of DEN induced foci reached ~500 foci/cm^3. This plateauing of foci may represent a carcinogen driven steady state between the initiation of foci forming hepatocytes and the loss of those hepatocytes through progression or cell death.

1270 THE EFFECT OF DNA REPAIR CAPACITY ON UV-A INDUCED MUTAGENESIS AND TRANSFORMATION, Harris S. Targovnik and William D. Bloomer, The Mount Sinai Medical Center, New York, NY 10029

Epidemiological data suggests that solar UV (290nm and greater) radiation is the primary etiological agent in skin cancer. The majority of the biological and biochemical effects observed are attributed to wavelengths in the solar UV-B region (290nm-320nm). The UV-A wavelengths of sunlight (320nm and greater) have previously been considered biologically unimportant due to their relatively low efficiency in inducing DNA damage. We have shown that UV-A radiation is far less efficient than short wavelength UV radiation in the induction of pyrimidine dimers. Moreover, UV-A radiation is capable of producing mutations at the HGPRT locus and transforming Balb/c 3T3 cells *in vitro*. This suggests that other DNA lesions and/or the inhibition of excision repair processes are also induced. We have also observed that UV-A wavelengths are inhibitory to pyrimidine dimer excision. This suggests a synergistic interaction between UV-B and UV-A wavelengths in the induction of mutagenesis and *in vitro* transformation.

1271 DETECTION OF BENZO(A)PYRENE DIOL EPOXIDE-DNA ADDUCTS BY SYNCHRONOUS FLUORESCENCE SPECTROPHOTOMETRY AND ULTRASENSITIVE ENZYMATIC RADIOIMMUNOASSAY IN COKE OVEN WORKERS, Kirsi Vahakangas*, Glennwood Trivers*, Aage Haugen**, William Wright***, and Curtis C. Harris*, *Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD 20205; **National Institute of Public Health, Oslo Norway; ***U.S.C. School of Medicine, Los Angeles, CA

Exposure to benzo(a)pyrene, a carcinogenic polycyclic aromatic hydrocarbon is followed by metabolism and covalent binding to macromolecules in animal and human tissues. The extent and importance of this binding in exposed human populations is not known. We are currently using synchronous fluorescence spectrophotometry (SFS) and ultrasensitive enzymatic radioimmunoassay (USERIA) in determining the DNA adduct level in peripheral white blood cells from exposed and non-exposed populations. In samples from 44 coke oven workers 25% (6/20) of the smokers, 21% (3/14) of the ex-smokers and 14% (1/7) of the non-smokers were positive by SFS. Similar distribution of positives between smokers and non-smokers were seen by USERIA. In similar material from 36 coke oven workers 4 (including 3 smokers) were positive. All of these were also strongly positive by USERIA. In addition, we detected 3 moderately and 8 weakly positive samples by USERIA. Samples from non-smoking laboratory workers were negative by both methods. The detection of carcinogen-DNA adducts indicates exposure to the carcinogen and its activation to a metabolite(s) that binds to DNA. The relationship between the amount of carcinogen-DNA adducts and cancer risk remains to be determined.

1272 Replicative Bypass Repair In Benzo[a]pyrene Diol Epoxide Treated, Synchronized CHO Cells, Doug Yamanishi and G. Tim Bowden, University of Arizona Medical School, Tucson, AZ 85724

A proposed model to account for chemical carcinogen mediated inhibition and recovery of DNA replication in mammalian cells is the "gap and block hypothesis." In this hypothesis if the carcinogen-DNA adduct is located on the template for the leading strand then fork movement is blocked and if the adduct is on the template for the lagging strand then a gap is formed because of the discontinuous mode of replication. We have obtained evidence for this model in synchronized Chinese hamster ovary (CHO) cells treated in late G_1 with (±) anti 7β, 8α dihydroxy-9α, 10α-epoxy 7,8,9,10 tetrahydrobenzo[a]pyrene (BPDEI) and allowed to proceed through the S phase. We have used BrdUrd labeling and CsCl gradient centrifugation as well as pH step alkaline elution assays to study the progression of BPDEI treated cells through S phase. Using doses of 0.33 μM and 0.66 μM BPDEI, the appearance of replicon size nascent DNA strands were delayed by 1-2 hr. and 2-3 hr., respectively; this indicated that replicon size DNA intermediates were blocked. The use of high resolution agarose gels to analyze Okazaki size nascent DNA intermediates and their relative abundance in control and BPDEI treated cells indicated that BPDEI treatment blocked the ligation of Okazaki intermediates in a dose dependent manner. The accumulation of replicon size intermediates is consistent with a block to the replication fork movement at DNA-carcinogen adducts and a block to Okazaki intermediate ligation is consistent with blocks to replication on the lagging strand leading to gap formation. Therefore, our data is supportive of the "gap-block" model of replicative bypass repair in mammalian cells. (Work supported by NIH Grant CA26972.)

Leukemia and Lymphoma

1273 GENETICS OF B-CELL NEOPLASIA, Carlo Croce, The Wistar Institute, 36th at Spruce Street, Philadelphia PA 19104

We have investigated the regulation of the expression of the c-myc oncogene involved in reciprocal translocations in Burkitt lymphoma by using somatic cell hybridization techniques. Results of this study indicate that different genetic elements are responsible for the in cis activation of the translocated c-myc oncogene in Burkitt's lymphomas with the t(8;14) translocation. We have also analyzed B-cell neoplasms of adults carrying the t(11;14) and the t(8;14) translocation and cloned the chromosome joinings on the 14q+ chromosome. These probes can be used to assess the rearrangements of the bcl-1 and bcl-2 in human B cell neoplasms.

1274 DNA ADDUCT FORMATION AND REMOVAL IN HUMAN CANCER PATIENTS, Miriam C. Poirier, Eddie Reed and Stuart H. Yuspa, National Cancer Institute, NIH, Bethesda, MD, 20205

cis-Diamminedichloroplatinum (II) (cisplatin) is a remarkably-potent chemotherapeutic agent, the cytocidal effects of which are caused by drug-DNA interactions. One such interaction results in the formation of an intrastrand deoxy(GpG)-N^7 bidentate adduct which, in cultured cells, comprises a major fraction of the total DNA-bound platinum and is slowly removed (1). We have elicited a polyclonal antibody specific for this adduct, and developed an Enzyme-Linked Immunosorbent Assay (ELISA) capable of detecting 25 attomoles of adduct/μg DNA or one adduct in 10^8 nucleotides (2). Because the antibody is specific for DNAs modified with certain chemotherapeutically-effective cis-reacting analogs of cisplatin, structurally-similar bidentate intrastrand adducts are believed to play a role in the tumoricidal efficacy of these compounds (3). Using the anti cisplatin-DNA-ELISA we have analyzed over 150 samples of DNA extracted from nucleated peripheral blood cells (buffy coat) of controls, and testicular and ovarian cancer patients at multiple times during cisplatin treatment. Of these, all 18 samples from untreated controls were never positive, and approximately 35% of samples from 45 cisplatin-treated patients were positive. Patients on their first course of chemotherapy receiving cisplatin on 21 or 28 day cycles (5 days of drug infusion followed by 2 or 3 drug-free weeks) readily accumulated DNA adducts as a function of dose. Patients on their first course of chemotherapy receiving cisplatin on 56 day cycles, or those given high doses after failing other chemotherapy, accumulated adducts much more slowly. Adduct accumulation in positive patients on 21 or 28 day cycles occurred both with total cumulative dose and increasing cycle number, suggesting that the removal time for measurable adducts formed during one cycle was more than 28 days. An analysis of disease response data for these patients suggested that individuals who did not form cisplatin adducts were less likely to respond to chemotherapy. It is anticipated that the ability to monitor a quantitative biologic effect of cisplatin exposure in the cancer patient may allow the clinician to objectively titrate cisplatin dose for chemotherapeutic efficacy while minimizing immediate toxicity and the threat of second malignancy. Concomitantly, studies utilizing the ELISA with human tumor cell lines and animal models may elucidate basic mechanisms of drug action and lead to more successful modes of treatment in the clinic.

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2) Poirier, M.C., Lippard, S.J., Zwelling, L.A., Ushay, H.M., Kerrigan, D., Thill, C.C., Santella, R.M., Grunberger, D. and Yuspa, S.H. Proc. Natl. Acad. Sci. U.S.A., 79: 6443-6447, 1982.
3) Lippard, S.J., Ushay, H.M., Merkel, C.M. and Poirier, M.C. Biochemistry, 22:5165-5168, 1983.

Detection of Oncogenes and Viruses in Human Tissues and Cells

1275 COLORIMETRIC IN-SITU HYBRIDIZATION OF PATHOGENIC VIRUSES IN FORMALIN FIXED TISSUES, David J. Brigati, Elizabeth R. Unger, David Myerson* and Lynn R. Budgeon, The Pennsylvania State University, Hershey, PA 17033 and *Fred Hutchinson Cancer Research Center, Seattle, WA 98108

Virtually all workers in the field of in-situ hybridization have reported decreased sensitivity in formalin fixed tissues compared to frozen tissues. Despite these difficulties, the ease of obtaining routinely fixed tissues, as well as the lure of the vast archives of case material already processed, makes optimizing conditions for formalin fixed tissues an attractive proposition. Routinely fixed autopsy tissues, including over-fixed tissues with formalin pigment and tissues on file as long as ten years, were used to investigate conditions for the in-situ hybridization of cytomegalovirus (CMV) and adenovirus. The result is a simplified method of in-situ hybridization yielding visual results within 8 hours. Specific histologic localization of viral genetic information has been achieved utilizing biotin labelled DNA probes followed by direct colorimetric detection with avidin-alkaline phosphatase complexes. We found significantly increased signal when the tissues and probe were denatured together at temperatures higher than those reported in previous methods. It is our suggestion that the increased temperature may be required in order to more fully denature cellular DNA which has been cross-linked by formalin. For CMV, positive results were achieved with a probe containing the putative transforming region comprising 1% of the viral genome. This simplified method of in-situ hybridization may be used in preliminary searches for viral transforming genes in human tumors.

1276 TRANSFORMING ACTIVITY OF HUMAN CYTOMEGALOVIRUS, F.M. Buonaguro, D.A. Galloway, C. Brandt and J.K. McDougall, Fred Hutchinson Cancer Center, Seattle, WA 98104

Human Cytomegalovirus has been implicated as a possible tumorigenic agent in a number of neoplastic processes - Kaposi's sarcoma, adenocarcinoma of colon, prostate cancer and cervical carcinoma. Evidence of the virus has been detected in such neoplasias with various success. In an attempt to elucidate the molecular mechanism for the oncogenic process we have been conducting transformation assays. NIH 3T3 cells have been transfected with a pBR322 recombinant plasmid containing a 558 bp fragment of HCMV strain AD169 previously shown to be oncogenic. Experiments have been conducted with and without cotransfection of a pSV2-neo plasmid that confers, as a dominant marker, the resistance to the antibiotic G-418. Morphologically transformed foci have been tested for anchorage independence and tumorigenicity in nude mice. DNAs extracted from primary transformants have been used for a second round of transfection. The pattern of integrated viral DNA sequences has been determined in some cell lines. Data from DNA sequences analysis and S1 mapping of RNA transcripts suggest that the transforming fragment does not encode any protein and its oncogenicity could be due to the ability to integrate into host DNA, that would in some rare instance result in a transformation event. To further explore the possibility that the HCMV transforming fragment can act as an insertional mutagen we have been testing its mutagenic activity at the hypoxanthine guanosine phosphoribosyl transferase (HGPRT) locus selecting for 6-thioguanine resistance. Preliminary experiments indicate that the HCMV fragment produces mutants and the mechanism of this activity is being studied.

1277 MOLECULAR ANALYSIS OF CHROMOSOME DAMAGE INDUCED BY ADENOVIRUS 12 AND ITS EFFECT ON ONCOGENES MAPPING IN THE DAMAGED REGION, Diane M. Durnam, Joan C. Menninger, Richard E. Gelinas and James K. McDougall, Fred Hutchinson Cancer Research Center, Seattle Wa 98104

Unlike other human adenoviruses, the highly oncogenic adenoviruses (eg. Ad 12) cause specific chromosomal damage in the human 17q21-q22 region and the p32-36,q12 and q42 regions of human chromosome 1. Abnormalities of these areas have also been associated with a variety of human neoplasias, ranging from acute promyelocytic leukemia to neuroblastomas, and are often associated with an increase in the severity of the disease. We are examining the mechanism of Ad 12 induced damage and the effect the damage has on oncogenes mapping in these regions. To determine which adenovirus gene(s) may be responsible for causing damage we are using a mouse-human fusion cell line (WL24A 2a) which has retained the 17q region as its sole human component and a recombinant Ad 5 (Rc15GT) which has the E1 region from Ad 12 in place of the Ad 5 E1 region (kindly provided by R. Bernard, J. Virol. 50:847 (1984)). Infection of WL24A 2a cells with either Ad 12 or Rc15GT results in specific damage in the human 17q region, which suggests that it is a gene in the Ad 12 E1 region which is responsible for the effect. We are also in the process of assessing changes in the structure and expression of oncogenes mapping in the damaged regions; these genes include the erb A oncogene in the 17q21-q22 region and the B lym and src genes in the 1p34-p36 region.

1278 DETECTION OF RARE HTLV-III-INFECTED CELLS IN PRIMARY TISSUE FROM AIDS PATIENTS USING A HIGHLY SENSITIVE IN SITU HYBRIDIZATION METHOD, Mary E. Harper, Lisa M. Marselle, Robert C. Gallo and Flossie Wong-Staal, NCI, Bethesda, MD 20205

We have detected HTLV-III RNA in a low percentage of primary lymphocytes from AIDS and ARC patients by in situ hybridization. High sensitivity of detection is possible by use of ^{35}S-labeled RNA transcribed from an HTLV-III/pSP64 DNA clone in combination with several hybridization pretreatment steps and post-hybridization ribonuclease digestion to prevent nonspecific labeling. Following two days autoradiographic exposure, HTLV-III RNA-containing cells exhibiting an average of 40 grains/cell were observed at low frequency (generally <0.01% of cells) in preparations from AIDS or ARC lymph nodes, peripheral blood, and bone marrow. Cell preparations from nonAIDS peripheral blood, nonAIDS bone marrow and the uninfected H9 T-cell clone were consistently negative. In contrast, H9 cells previously infected with HTLV-III were highly labeled (30-200 grains/cell) when hybridized with the HTLV-III probe. Also, hybridization of a probe control, ^{35}S-RNA specific for λ DNA, to AIDS cells or the H9/HTLV-III cell line resulted in no label. These results indicate that in situ hybridization can be used to detect primary cells expressing HTLV-III RNA after short exposure. Grains observed are specific for viral RNA and are present in a significant percentage of cases so far examined. It can also be concluded from these and Southern blot experiments that the number of HTLV-III-infected cells in AIDS or ARC primary tissue is very low and that lymph node enlargement in patients with lymphadenopathy is not due to a proliferation of HTLV-III-infected lymphocytes.

Neoplasia of the Hematopoietic System and Reproductive Tract; Other Cancers

1279 TRANSFECTION OF CHORIOCARCINOMA DNA AND METHYLATION, Saverio Alberti and Leonard A. Herzenberg, Stanford University, Stanford, CA 94305

Recently, we transfected lymphocyte markers in mouse L cells using as sources of DNA a number of tissues that do not express these antigens (1). We have shown that trophoblast cells are unique in their lack of expression of HLA class I antigens (2). This is probably important for the survival of the fetus as implant and may be relevant for the growth of trophoblast tumors (choriocarcinomas). Thus we have investigated the expression of HLA class I antigens and of the lymphocyte markers Leu-1 and Leu-2 upon transfection of DNA from 4 choriocarcinoma cell lines. These cells differ in their levels of HLA expression from undetectable to very high. Interestingly, the DNA from neither Jar nor Bewo, cells with undetectable and low HLA expression, transfected HLA, Leu-1 or Leu-2. DNA from Ima, a low expressing HLA line, transfected HLA but not Leu-1 or Leu-2 while DNA from Enaml, which is highly positive for HLA, tranfected all the markers studied. All cell lines efficiently transfected Trop-1, a surface molecule expressed by choriocarcinomas. Southern blots demonstrated that all cell lines contain the genes coding for HLA and Leu-2 and that these genes are indistinguishable from those of leukocytes, using a number of different enzymes. However, using the isoschizomers Hpa II and Msp I the patterns of methylation of HLA and Leu-2 genes from the various sources differed. Thus, differential DNA methylation may play a role in the expression of the genes studied.

1) Nature, 312: 68 (1984).
2) J. Exp. Med., 160: 633 (1984).

1280 UNRESTRICTED (H-2) GROWTH OF A MALIGNANT LYMPHOBLASTIC TUMOR OF MOUSE ORIGIN, Gabriel Nava, Enrique Escandón, Veronica Yakoleff and Guillermo Alfaro, National University of México. México D.F. 04510, México.

Murine tumors are generally restricted (H-2) when grown in mice of a different haplotype. However, in some cases it is possible to isolate cell variants which are capable of overcoming that restriction and proliferate across halogenic barriers. We have identified a cell line (LRK), originally thought to be L5178Y which has the capacity of growing in mice of one of various haplotypes(H-2b,d or k) without any detectable differences either in the capacity to produce metastasis (usually very poor) or the time required to kill the mice. This cell line doesnot express MHC molecules in its membrane as determined by the use of antibodies against H-2 and complement or with cytotoxic T lymphocytes. Cell hybrids were constructed between appropriate mutants of LRK and another lymphoblastic tumor (LW) in order to investigate the expression of H-2 and other cellular properties. The experimental strategy included the determination of electropherotypes of metabolically labelled proteins in one and two dimensional gels; the implementation of a protocol to explore the immunological properties, and the capacity to propagate these new cells in mice of one of various haplotypes.

1281 A POTENTIAL MECHANISM OF TRANSFORMATION BY HERPES SIMPLEX VIRUS, TYPE 2, DNA, Bruce Bejcek and Anthony J. Conley, St. Louis University Medical Center, St. Louis, MO. 63110

The mechanism for HSV-2 transformation is not known. Two distinct morphological transforming regions (mtrII and mtrIII) have been identified. We transformed rat cells with mtrII DNA fragments. The cells contain multiple copies of viral DNA integrated in a complex manner. One line also contained a 20 Kbp extrachromosomal element. Remarkably, this element is able to rapidly transform cells at a 3000-fold higher frequency than mtrII DNA alone. This element contains rat sequences that are homologous to a known cellular oncogene. These same sequences are homologous to a subset of mtrIII DNA. Further, the element contains two separate copies of a small portion of the input transforming DNA. This small portion contains the type-specific direct repeat region DNA reported by Galloway and colleagues to induce foci. One copy is near the rat oncogene-mtrIII sequences. We suggest that the activation of the oncogene sequences is responsible for the rapid and high frequency transformation capacity of this element. We speculate that the structure of this element reflects a general mechanism of HSV-2 induced transformation: i.e. the activation of a cellular oncogene by the HSV-2 direct repeat region DNA.

1282 METASTASIS IN NUDE MICE OF CELLS CONTAINING VARIOUS Ha-ras GENES AND THEIR RECOMBINANTS, Matthews O. Bradley, Andrew Kraynak, and Jackson B. Gibbs, Merck Sharp and Dohme Research Laboratories, West Point, PA 19486

We are studying the metastatic behavior of different cell types containing a variety of activated oncogenes. As a first step, we have developed models of tumor embolization and spontaneous metastasis in nude mice that are also NK cell deficient. Our initial studies have compared the ability to form tumor nodules in lungs after tail vein injection of NIH 3T3 cells that contain 4 different ras oncogenes. The p21 proteins produced by these genes are: the normal cellular ras gene, EC(Gly^{12},Ala^{59}); the activated oncogene, EJ (Val^{12},Ala^{59}); a recombinant gene, EC/v-Ha(Gly^{12},Thr^{59}); and a second recombinant gene, EJ/v-Ha(Val^{12},Thr^{59}). The results show that 3T3 cells containing these genes readily form lethal tumor nodules in the lungs of nude mice after tail vein injection. No control 3T3 cells formed tumors. The most embolic cell contained the EJ/v-Ha recombinant whose protein has the lowest GTPase activity, but the highest autophosphorylating activity. Interestingly, cells containing the EC gene on an LTR were also able to form experimental metastases. These studies show first, that metastatic embolization can be conferred simply by increased expression of normal cellular p21,and second, that lower levels of GTPase activity along with higher levels of autophosphorylation in mutant p21s correlate with increased embolization.

1283 STUDIES ON THE MECHANISM OF TRANSFORMATION AND MUTATION BY HSV-2, C. Brandt, F. Buonaguro, J.K. McDougall and D. Galloway, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

We have recently shown that a 737 nucleotide fragment (BC24) of HSV-2 can morphologically transform cells when transfected into NIH 3T3 cells.[1] The nucleotide sequence of this fragment reveals a potential stem loop structure with short direct repeats at the base of the stem. This stem loop flanked by direct repeats is reminiscent of bacterial insertion sequences. Because HSV-2 does not encode a transforming protein, the transforming potential of the virus may reside in its ability to disrupt cellular gene expression. We are currently screening transformed cell lines for the presence of integrated HSV-2 DNA.

As a complementary test of the insertional mutagenesis hypothesis, we are also examining the mutagenic activity of the HSV-2 BC24 fragment with a selectable marker. NIH 3T3 cells have been transfected with BC24 and plated in 6-thioguanine to select for resistant mutants. Seven 6-thioguanine resistant mutants have been isolated and are currently being analysed for the presence of BC24 sequences and alterations in the HGPRT gene. Additional experiments to quantitate the mutagenic potential of the HSV transforming fragment and to optimize the mutation assay are in progress.

[1]Galloway et. al. (1984) P.N.A.S. 81, 4736.

1284 ISOLATION OF A HUMAN ENDOGENOUS RETROVIRUS (ERV3) cDNA CLONE FROM A 20 WEEK HUMAN FETAL LIBRARY, Maurice Cohen[1], Susan Pfeifer-Ohlsson[2], Rolf Ohlsson[2], Marilyn Powers[1], Jan Rydnert[3], and Catherine O'Connell[1], [1]Laboratory of Molecular Virology and Carcinogenesis, LBI-Basic Research Program, NCI-Frederick Cancer Research Program, Frederick, MD 21701, [2]Deparment of Oncology, University of Umeå, Umea, Sweden, [3]Department of Gynecology, University of Umeå, Umeå, Sweden

Endogenous retrovirus 3, or ERV3, is a 9.9 kb endogenous human provirus that was isolated from a human recombinant DNA library cloned in λ Charon 4A by low stringency hybridization using two retroviral probes: BaEV LTR, and an endogenous chimpanzee retroviral clone resprepresenting the 3' end of the pol gene. This full length provirus may be defective due to in-frame termination codons in its gag and pol genes. The env gene, however, contains an open reading frame spanning more than 1.8 kb. The ERV3 provirus was mapped to a single genomic locus on human chromosome 7. The DNA sequence of the ERV3 LTRs revealed them to contain apparently normal promoter sequences and two of the hexanucleotide sequences upstream of the promoter that are responsible for binding activated glucocorticoid receptor in the MMTV LTR. We find ERV3 env-specific transcripts of 4 kb and 2.2 kb in RNA isolated from human first-term placenta and fetus. Further, from a 20 week human fetal cDNA library, we isolated two cDNA clones using the ERV3 env region as probe. DNA sequencing of one clone revealed that its sequence is identical to that of the ERV3 proviral genome. (Supported by NCI Contract NO1-CO-23909).

1285 THE ROLE OF HUMAN CYTOMEGALOVIRUS (HCMV) IN TUMOUROGENESIS AND POSSIBLE INVOLVEMENT IN THE DEVELOPMENT OF CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN), K. Fletcher[1], J. Cordiner[2] and J.C.M. Macnab[1]. [1]Institute of Virology, Glasgow, [2]Department of Midwifery, Queen Mothers Hospital, Glasgow.

We have been investigating the role of HCMV in tumourogenesis by examining both CIN biopsies and rat embryo cells oncogenically transformed by HCMV. DNA has been extracted from CIN biopsies and transferred to biodyne filters by the method of Southern. The DNA was hybridized to a Hind/Bam subclone of the AD169 Hind III E fragment which contains the transforming region (Nelson, 1982) HCMV DNA sequences have been found to be present at 10-20 copies per cell in one CIN biopsy. No HCMV DNA sequences have been detected in other CIN biopsies of our series at a level of 1 copy per cell. We have also formed an in vitro model system to study HCMV oncogenesis. Rat embryo cells have been transformed with UV inactivated HCMV AD169. DNA extracted from transformed cells, tumours and cell lines cultured from the tumours has been probed with the transforming region and no HCMV DNA sequences have been found to be present at 1 copy per cell. These results suggest that HCMV DNA may only be necessary to initiate transformation and not required for maintenance of the transformed state. The roles of HCMV in inducing host cell proteins and latent infections, events which may be involved in a multistage carcinogenic process, are at present being evaluated.

1286 MYELOID PRECURSORS CONTAIN LOW LEVELS OF O^6 ALKYLGUANINE ALKYLTRANSFERASE ACTIVITY S. L. Gerson, K. Ray, and N. A. Berger, Case Western Reserve, Cleveland, Ohio 44106

O^6 alkylguanine alkyltransferase (O^6G alk-trans) repairs the promutagenic adduct, O^6 alkylguanine. Since alkylating agent exposure is linked to acute myeloid leukemia, we studied O^6G alk-trans activity in myeloid cell lines, blood cells and marrow myeloid precursors. O^6G alk-trans activity was determined by the ability of cell extracts to remove [^{3}H]O^6 methylguanine (O^6MG) from methylated DNA during a 60 min inc at 37°C as quantified by HPLC separation of O^6MG following DNA acid-hydrolysis. O^6G alk-trans activity was linear relative to the protein concentration of cell extract and the reaction was complete by 30 min. O^6G alk-trans activity, reported as fM O^6MG removed/mg protein varied greatly amoung three myeloid cell lines: HL-60 (373±24), KG-1 (221±36) and K562 (47±22). In peripheral blood from 11 donors, the O^6G alk-trans activity was 89.4±10.7 in granulocytes, 162±254 in monocytes, 189±17 in T lymphocytes and 139±29 in B lymphocytes. In bone marrow from 17 normal donors, the O^6G alk-trans activity was 210±27 in T lymphocytes and 143±19 in myeloid precursors (myeloblasts through myelocytes) ($p<0.05$, paired t-test). O^6G alk-trans activity in myeloid precursors was 3-8% of O^6G alk-trans activity in normal human liver and 12-18% the level found in normal human intestine, normalized to the DNA concentration of the cell extract. Low levels of O^6G alk-trans in myeloid precursors may allow persistence of O^6 alkylguanine adducts and predispose these cells to leukemic transformation following alkylating agent exposure.

1287 TREATMENT OF A FATAL TRANSPLANTABLE ERYTHROLEUKEMIA BY PROCEDURES WHICH LOWER ENDOGENOUS ERYTHROPOIETIN, W. David Hankins and Azhar Hossain, National Cancer Institute, Bethesda, MD 20205

We previously reported that some, but not all, erythroid cell lines derived from leukemic infected mice produced erythropoietin (Epo). To better understand the relation of Epo and erythroleukemia development, we derived 7 additional erythroleukemic lines which induce a fatal leukemia when transplanted in vivo. In vitro culture of leukemic spleen cells revealed that three different preparations of Epo dramatically stimulated their proliferation. The Epo-responsive cells retained their leukemogenic potential when reinoculated in vivo. These in vitro results led us to examine the influence of Epo on tumor growth in vivo. Transfusion of RBC's prior to or after inoculation significantly retarded leukemia development and greatly extended the survival of inoculated mice. While other explanations are possible, it is likely that the transfusion effects were mediated through reduction of endogenous Epo since simultaneous administration of Epo led to rapid splenomegaly and death. These data indicate that (A) transplantable erythroleukemia cells retain sensitivity to Epo, (B) antihormone therapy may be efficacious in this fatal leukemia, and (C) even in the absence of apparent differentiation Epo stimulated proliferation of leukemic cells.

1288 COLONY FORMATION BY BONE MARROW CELLS FOLLOWING TRANSFECTION OF CLONED ONCOGENES, H.Herbst+, C.LeFevre+, J.S.H.Wong*, B.A.Roe*, and M.J.Cline+, +Div. of Hematology-Oncology, UCLA School of Medicine, Los Angeles, CA 90024, *Dept. of Chemistry, University of Oklahoma, Norman, OK 73019

In an attempt to study the role of oncogene sequences in leukemogenesis we have constructed non-retroviral eukaryotic expression vectors for the oncogenes v-abl, v-kis, v-myb, and c-myc involving promoters from the SV40 early region, metallothionein, and mouse mammary tumor virus LTR. Additional eukaryotic gene processing signals were provided by a 3' fragment of the rabbit ß globin gene. In a second set of vectors these 3' signals were substituted by a retroviral LTR. Upon transfection of linearized vector DNA employing Ca/PO4 precipitate and glycerol shock techniques into murine bone marrow cells induction of colony formation in soft agar could be observed in the absence of exogenous colony stimulating factor. Some colonies were apparantly immortalized and exhibited prolonged growth. The frequency of colony formation, growth behaviour of individual colonies, and other parameters were dependent on the oncogene(s) transfected, promoters, and 3' signals. Using equal amounts of DNA, the highest rate of colony formation could be obtained with v-kis and v-abl employing SV40/ß globin vectors. In case of modulatable promoters, colony formation was dependent on appropriate induction. Furthermore, the rate of colony formation was highly augmented by a 3' LTR, probably through the action of retroviral enhancers of transcription. We are currently investigating the phenotype of cells in colonies of clearly immortalized cells and colonies that cease growing within weeks after transfection. The significance of these observations in comparison to recently published experiments with recombinant retroviruses will be discussed.

1289 GENETIC CHANGES IN PRELEUKEMIA AND LEUKEMIA, Russel E. Kaufman, Joni Nichols, Frank Deal and Cynthia Chua, Duke University, Durham, NC 27710

Chemotherapy related leukemia is frequently preceded by a preleukemic phase. We have attempted to identify patterns of oncogene activation during the preleukemic phase and to identify changes in this pattern of activation during progression to acute leukemia. We have examined the K-ras oncogene for mutations in codon 12, and have examined the myc oncogene to determine copy number. In our study of eleven patients, we demonstrate that one patient has a mutation in the 12th codon of K-ras to produce a new Sac 1 restriction site. No patients have amplification of myc in the preleukemic phase, although two patients have increased copy number following transition to leukemia. We conclude that the pattern of oncogene activation changes in some patients as they progress to leukemia.

1290 AMPLIFICATION OF c-myc ONCOGENE IN ACUTE MYELOID LEUKEMIA CELLS WITH dmin CHROMOSOMES. Jorma Keski-Oja, Kalle Saksela, Riitta Alitalo, Robert Winqvist, Marikki Laiho, Albert de la Chapelle and Kari Alitalo, Department of Virology, and III Department of Medicine, University of Helsinki, Haartmank. 3, 00290 Helsinki 29 FINLAND.

Cytogenetic analysis of the bone marrow cells of a 52-year old woman with AML showed cells with trisomy of chromosome number 4. The proportion of abnormal karyotypes was lower in subsequent analyses but virtually all mitoses from bone marrow contained an extra chromosome 4, and numerous (20-50) double minute chromsomes. Part of the mitoses also had trisomy of chromosome number 6.
DNA extracted from the bone marrow cells (containing dmin) was analyzed for the amplification or rearrangement of different oncogenes using twelve different oncogene probes including ras, src, myb, myc. Evidence for rearrangements was not obtained. About ten-fold amplification of an apparently nonrearranged c-myc gene was found, whereas the other probes identified single gene copies in the genome.

This is, to our knowledge, the first case where amplification of a cellular oncogene (c-myc) is detected in AML cells fresh from the patient. Previously, promyelocytic leukemia cells (HL-60) were found to have some dmin:s and amplified c-myc when freshly isolated from the patient.

1291 PHARMACOKINETIC ANALYSIS OF dsRNA THERAPY IN CANCER: CAVEAT OF CLONOGENIC ASSAYS. L.J. Krueger, D.R. Strayer, P.J. Andryuk, G.L. Kieffer and W.A. Carter. Department of Hematology/Oncology, Hahnemann University, Philadelphia, PA 19102. Ampligen®, a double-stranded mismatched RNA molecule, poly(I)·poly(C_{12}U), causes substantial biological damage to cancer cells. A major benefit of Ampligen® therapy has been the absence of significant side effects, especially when compared to the high percent of patients who experienced unacceptable toxicity with poly(I)·poly(C). The toxicity of poly (I)·poly(C) or its derivatives occurred either through drug toxicity or by the delayed production of neutralizing antibodies. In vitro studies have shown that the reduction of toxicity found during Ampligen® treatment may be attributed to its accelerated decay. However, it has been difficult to quantify nucleic acid concentrations in human blood samples. We report the modification of the Quickblot® nucleic acid immobilization and hybridization technique (Bresser et al. (1983) Proc. Natl. Acad. Sci. USA 80, 6523-7) to quantify sera levels of Ampligen® in vivo. This assay has a sensitivity of as little as 30 ng/spot and can be used to measure drug levels over several orders of magnitude. In 7/8 patients measured to date, Ampligen could be detected at least 2 hr after beginning the drug infusion. Measurements of Ampligen decay suggest a half-life varying from 15-90 min. However, in one patient, circulating Ampligen® levels could not be detected even during infusion. Although fresh biopsy material was found to be most sensitive to Ampligen®, other factors, i.e. serum RNAse levels, may influence clinical responsiveness.

1292 TRANSFECTION AND REGULATED EXPRESSION OF A TOXIN GENE IN HUMAN TUMOR CELLS, Ian H. Maxwell, L. Michael Glode and Francoise Maxwell, Division of Medical Oncology, University of Colorado Health Sciences Center, Denver,CO 80262

We are attempting to demonstrate cell killing as a result of the endogenous expression of a transfected toxin gene and to achieve tissue-specificity of expression, as a possible novel approach to cancer therapy. We have constructed plasmids containing the diphtheria toxin mature A chain (DT-A) coding sequence linked to an inducible metallothionein promoter and have used these constructs to transfect HeLa cells, together with the neo selectable marker in a separate plasmid. Similar constructs containing the cat gene, or mutant DT-A genes, are being used in control experiments. We shall report on the effects of induced expression of these genes in stable co-transformants selected for G418 resistance. Experiments designed to direct specific expression of these genes in transfected B lymphoid cells will also be presented. (Supported in part by grant No. 84-37 from the Milheim Foundation for Cancer Research.)

1293 INTERACTION BETWEEN RAF AND MYC ONCOGENES IN TRANSFORMATION IN VITRO AND IN VIVO, John L. Cleveland, Herbert C. Morse, James N. Ihle and Ulf R. Rapp, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701

We have investigated the transforming activities of the raf and myc oncogenes in hematopoetic and fibroblastic cells using a series of oncogene-transducing murine retroviruses. 3611 MSV, containing the raf oncogene, induces fibrosarcomas in newborn mice after a latency of 4-8 weeks. In contrast, newly constructed myc oncogene-transducing murine retroviruses induce lymphomas after a 9-week latency. A combination of both oncogenes in an infectious murine retrovirus induces hematologic neoplasms 1-3 weeks after inoculation; these neoplasms primarily consist of immunoblastic lymphomas of T and B cell lineage and erythroblastosis. Cells from tumors induced by the myc- or raf + myc-transducing viruses can be readily established in culture in regular medium whereas culture of cells from raf oncogene-induced tumors requires the addition of IL3. Infection of a series of IL2 and IL3-independent myeloid leukemia cell lines with the various viruses has demonstrated that recombinants expressing myc abrogate growth factor requirements of IL2- or 3-dependent infected cell lines. In parallel to the synergistic action of raf and myc oncogenes on hematopoeitic cell transformation in vivo, we find that raf oncogene-induced transformation of fibroblast cell lines in culture is enhanced by the addition of myc which by itself does not morphologically transform these permanent cell lines. We conclude that concomitant expression of raf and myc oncogenes in hematopoetic and fibroblast cells and cell lines enhances their respective transforming activities.

1294 **THE IMMUNODEFICIENCY CANCER REGISTRY (ICR).** LL Robison, G Frizzera, AH Filipovich. From the Departments of Pediatrics, Laboratory Medicine and Pathology. University of Minnesota Hospitals, Minneapolis, MN 55455.

The ICR is a worldwide registry currently consisting of 468 cases of cancers in patients with naturally-occurring immunodeficiency disorders (ID). The ICR collects tumor material for standardized pathology review as well as to form a repository of material for future biochemical and molecular studies. The ICR contains a disproportionately high percentage of lymphomas including non-Hodgkin's lymphomas (NHL) (43%) and Hodgkin's disease (HD) (7%), most occurring at a younger age than expected. NHL are the predominant tumors reported in ID with partial T-cell dysfunction: Wiskott-Aldrich syndrome (WAS) 64% (47/74) ataxia talangiectasia (A-T) 42% (61/145), common variable immunodeficiency 38% (43/114) and severe combined immunodeficiency (SCID) 70% (26/37). In contrast to NHL in non-ID patients, WAS patients were found more frequently to have: histology of histiocytic lymphoma, CNS involvement and extranodal disease. Detailed review of clinical and tumor material from 21 WAS patients demonstrated that 16/21 tumors (diagnosed at a mean age of 6.7 years) had the histologic appearance of B cells in multiple stages of transformation. As evidence of immunoregulatory dysfunction, autoimmune reactions preceded B cell NHL in 6/16. The ICR contains 31 cases of HD diagnosed at a mean age of 11 years: 13 A-T, 3 WAS, 1 SCID, and 10 variable immunoglobulin defects. Unlike HD in the general population, unfavorable histologies were found in 45% of cases and included lymphocyte depletion and mixed cellularity. Additional evaluation of such ICR cases may provide data to assist in the identification of predisposing risk factors for lymphoma.

1295 STEROID HORMONES AND THE DEVELOPMENT OF FEMALE REPRODUCTIVE CANCERS: EFFECTS OF POTENCY AND DURATION, Elaine M. Smith, University of Iowa, Iowa City, IA 52242

The effects of oral contraceptives and postmenopausal steroids were examined to determine how reproductive cancer risk varied with pharmacologic potency (progestational and estrogenic effects on target tissues) and duration of hormone use. Personal interviews of women with cancer of the breast (N=580), endometrium (N=101), and ovary (N=83), ages 20-54, identified from the Iowa State NCI population-based cancer registry, and 716 age-frequency matched controls, reviewed lifetime history of specific brands and dosages of steroid hormones, and other cancer risk factors. Oral contraceptive (OC) potency was evaluated using the Ferin Assay (FA); and postmenopausal steroid (PMS) potency using suppression of FSH Assay. There was no significant association between OC potency x duration (all OC combined) and risk of breast or endometrial cancer. OC users had a reduced risk of ovarian cancer (RR=.86, $p<.005$), regardless of potency or duration. No significant association was found between PMS (all PMS combined) and risk of cancer. Logistic models of pharmacologic potency effects showed a reduced risk of ovarian cancer with low potency (RR=.79/yr.) and mid-level potency (RR=.88/yr., $p<.05$) OC progestins. Sequentials (RR=1.48/yr., $p<.01$) and higher FSH PMS potency drugs (RR=1.32/yr.) increased the risk of endometrial cancer, while high progestin potency drugs (RR=.82/yr., $p<.05$) decreased the risk. Modeling of steroid hormones for lifetime and for potency x duration effects on cancer risk also was assessed.

1296 MOLECULAR ANALYSIS OF THE CHROMOSOMAL CONTROL OF NEOPLASTIC EXPRESSION IN HUMAN CELL HYBRIDS, E. S. Srivatsan and E. J. Stanbridge, University of California, Irvine, California 92717

Correlation of the loss of specific chromosomes with the expression of tumorigenicity in intraspecific human hybrid cells was studied. Human hybrid cell lines derived from HeLa X fibroblast were analyzed using restriction fragment length polymorphisms (RFLPs). Genes for β-globin, C-Ha-ras-1 and insulin on chromosome 11 and a polymorphic locus AW101 on chromosome 14 were used as Southern hybridization probes. Analysis of DNA from fibroblast, HeLa and their non-tumorigenic and tumorigenic hybrids showed the loss of a fibroblast chromosome 11 in three tumorigenic hybrids and a HeLa chromosome 11 in a fourth tumorigenic hybrid cell line. This latter hybrid has also lost a copy of chromosome 14 of fibroblast origin. Normal chromosomes 11 and possibly 14 thus seem to play an important role in the control of neoplastic expression in the human hybrid cell system.

1297 DNA PROBES AS GENETIC MARKERS IN CANCER STUDIES, Scott R. Woodward, Dora Stauffer and Ray White, Howard Hughes Medical Institute, Salt Lake City, Utah 84132

The power of restriction fragment length polymorphisms analysis to aid in the elucidation of the mechanisms involved in retinoblastoma and Wilms tumor has been recently demonstrated. These techniques are now being advanced to understand underlying genetic mechanisms in breast cancer, multiple endocrine neoplasia, neurofibromatosis and colon cancer. Familial polyposis coli families are being used as a model system for colon cancer. Adenomatous polyps and colon cancers are being tested for reduction to homozygosity on each chromosome using oncogenes and random DNA polymorphisms. Breast cancer, multiple endocrine neoplasia and neurofibromatosis families are being tested for linkage using various DNA polymorphisms.

1298 APPEARANCE OF A 14q+ MARKER CHROMOSOME IN A HUMAN B-CELL LINE IN CULTURE, David W. Yandell and John B. Little, Harvard School of Public Health, Boston, MA 02115

The 14q+ marker chromosome is one of the most specific chromosome abnormalities seen in human neoplasia. It is most commonly seen in neoplasms of a pre-B or B-cell origin; in Burkitt Lymphoma this marker appears as a result of translocation of the c-myc gene into the immunoglobulin heavy chain locus.

We have investigated the appearance of a 14q+ marker chromosome in a human B-lymphoblastoid line in culture. This cell line is of special interest because it was derived 16 years ago from an individual apparently free from any hematopoietic malignancy. No marker chromosome is apparent in chromosome spreads prepared 1 year after the line was initiated in culture. Since that time, the line has been grown extensively in vitro and now grows rapidly with an extraordinarily high cloning efficiency. Cytogenetic analysis shows the breakpoint on chromosome 14 to be at or near the immunoglobulin heavy chain locus at 14q32. Molecular analysis shows that this cell line carries two rearranged heavy-chain alleles but unrearranged lambda-light chain loci. Thirteen onc-genes most commonly associated with human malignancy, including c-myc, were tested for rearrangement but all showed a normal arrangement when compared to diploid human control cell lines. Further studies are underway to determine whether other onc genes or endogenous cellular sequences are involved in this rearrangement.

1299 ALTERATIONS IN T CELL FUNCTIONS BY PBL FROM POSTMENOPAUSAL WOMEN WITH ENDOMETRIAL HYPERPLASIAS AND CARCINOMA-POSSIBLE ROLE OF ESTROGENS, *Ilana Yron, *Michael Schikler, +Jardena Ovadia and *Isaac P. Witz.
*Tel Aviv University, Tel Aviv, Israel. +Beilinson Medical Center, Petach-Tikva, Israel.

Alteration in IL-2 production and cell proliferation following PHA stimulation of peripheral blood lymphocytes (PBL) from non-invasive stage I Endometrial carcinoma (E.C.) patients, and the mechanisms involved have been studied. We have been using this tumor system as a model for studying the in vivo effect of over exposure to estrogens on T cell responses. Since we include in our study only non-invasive stage I E.C. patients before any treatment it is likely that T-cell disfunctions, if detected, would be influenced by their special hormonal set up rather than being a secondary effect of the malignant process itself. Our study thus included 3 groups, E.C. patients, postmenopausal women at high risk for developing E.C. and age matched healthly women. IL-2 production was markedly lower in most E.C. patients as compared to healthy controls. Varying levels of IL-2 were produced by PBL from women from the high risk group. PBL proliferative response to PHA appears to correlate with levels of IL-2 production. Preliminary experiments suggest that macrophages as well as T suppresosr cells may be involved in modulating patients' T cell functions. In vitro assays studying the inhibitory effect of estrogens on T cell regulatory functions and the mechanisms involved will also be presented.

1300 ONCOGENE EXPRESSION IN RADIATION INDUCED RAT THYROID CARCINOMA. Zain, S., Mulcahy, T., and Kaminsky, S. Cancer Center, University of Rochester, Rochester, N.Y.

To evaluate the hypothesis that oncogene expression occurs as a step in radiation carcinogenesis, we used a rat thyroid radiation carcinogenesis model capitalizing on the exquisite sensitivity of thyroid epithelium to the carcinogenic effects of ionizing radiation. Tumor cells derived from radiation induced thyroid carcinoma in early passage were used as a source of tumor material. Comparison between the genomic DNA and mRNA transcripts between the normal thyroid and radiation induced carcinoma revealed the expression of a set of oncogenes (myc, Ras^k, abl) which qualitatively differs from that of the normal. In addition, the DNA from the tumor tissue gives indications of DNA rearrangements (myc) and gene amplification (Ras^k).

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 transformation of thyroid epithelial cells will be discussed.

Cancers of the Reproductive Tract

1301 MOLECULAR EPIDEMIOLOGY AND ONCOGENESIS OF HUMAN CYTOMEGALOVIRUS. Eng-Shang Huang and Michelle G. Davis, Cancer Research Center, and Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514.

Human cytomegalovirus (CMV), is an ubiquitous herpes virus. It has been implicated as a possible oncogenic agent due to its ability to stimulate cellular micromolecules synthesis and to morphologically transform rodent and human cells. To understand the molecular epidemiology of CMV, we have applied DNA restriction enzyme (RE) analysis to study virus strains isolated from groups with various epidemiological identity. Our results indicate (a) CMV has remarkable heterogeneity; various CMV isolates have distinct RE patterns. Considerable comigrating fragments are found among various isolates, however no two epidemiologically unrelated isolates have identical RE patterns. (b) The majority of recurrent maternal infection represents reactivation of existing latent viruses, however reinfection by a new virus does occur ococasionally. (c) Virus isolates from mother and offspring, and from consecutive congenitally infected infants born to the same women are genetically related. (d) CMV can persistently infect individuals for a substantial period of time without altering its RE marker. (e) Post-transplanted CMV infection is frequently caused by a CMV strain genetically identical to the pre-transplant isolate. (f) Strains isolated from patients with AIDS are not identical, and finally (g) genetically related strains can be isolated from semen of homosexual men residing in the same area.

As with other DNA tumor viruses, human CMV is found to stimulate the synthesis of cellular DNA, RNA, mitochondria DNA and enzymes related to cell proliferation: such as DNA polymerases, RNA polymerase, thymidine kinase, ornithine decarboxylase and plasminogen activator. As demonstrated by several investigators CMV and its DNA fragments were able to transform hamster embryonic cells, NIH3T3 as well as human fibroblasts. In human fibroblast systems, we found that subfragment of largest CMV HindIII fragment was able to transform human embryonic lung cells. This fragment could hybridize to 1.5 kb v-myc DNA at highly stringent hybridization condition. But the removal of high G+C portions of DNA from v-myc DNA render negative hybridization results. This implies the former report of the existence of myc related sequence in CMV might be due to the existence of high GC containing DNA in this area. By nucleic acid hybridization and various immunological techniques, we have observed that CMV DNA and virus-specific macromolecules frequently exist in various human tumor tissues; these include cervical cancer from the Far East, prostatic adenocarcinoma and ovarian cancer from the states, and Kaposi's sarcoma from Africa. CMV is commonly existent in urogenital organs, thus, it is difficult to confirm the etiological role of CMV with these cancers. Nevertheless, its oncogenic potential as demonstrated in vitro suggest that CMV might have oncogenic potency in urogenital organs.

1302 The Role of Papillomaviruses in Tumors of the Urogenital Tract.
Harald zur Hausen, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg 1

At least five distinct types of papillomaviruses have recently been identified in the human genital tract. Human papillomavirus (HPV) type 6 is found in about 60 % of condylomata acuminata as well as in a small percentage of cervical condylomatous lesions. HPV 11 is detected in approximately 30 % of additional condylomata acuminata and in about 50 % of koilocytotic dysplasias of the cervix. Some of the latter lesions also contain HPV 10. Two virus types, HPV 16 and HPV 18 were recently identified in about 70 % of invasive cervical cancer biopsies, as well as in vulval and penile cancer. At least about 15 % of additional biopsies contain HPV-cross-hybridizing sequences, presumably originating from yet non-identified papillomaviruses. HPV 16 is frequently found in atypical dysplasias, in carcinomata in situ and in about 80 % of genital Bowen's disease and Bowenoid papulosis. HPV 18 DNA has been identified in cells of several lines originating from cervical cancer, including the HeLa line. The etiological involvement of these viruses in genital malignant tumors and their possible synergism with tumor-initiating factors will be discussed.

Late Additions

1303 Human T-Cell Leukemia Viruses in Leukemia and Immunosuppression.
M. Essex, Department of Cancer Biology, Harvard School of Public Health, Boston, MA 02115

Members of the human T-cell leukemia virus (HTLV) family of agents have been linked to adult T-cell leukemia (ATL), to AIDS, and to a form of clinical immunosuppression that is less severe than AIDS.[1] Viruses of the same family have been detected in numerous species of Old World monkeys, in some cases in association with outbreaks of lymphoma and/or immunosuppression.[2]

The HTLV proteins most appropriate for seroepidemiologic screening of populations exposed to HTLV I, HTLV II, and HTLV III are the *env* and *lor* gene products.[3,4] The major *env* gene product, which is designated gp61 in the case of HTLV-I, gp67 in the case of HTLV-II, and gp120 in the case of HTLV-III, is the most immunogenic viral protein in exposed people and antibodies to this class of protein are found in essentially all ATL patients or AID patients. The major *lor* gene product, designated p42 for HTLV-I and p38 for HTLV-II is immunogenic in some individuals, localized in the nucleus, and its expression may be related to immortalization. The presence of antibodies to the *env*, *lor*, and other viral gene products provide a mechanism to monitor populations for exosure to HTLV's as well as to estimate disease risk in exposed individuals.

1. Essex, M. *In* Human T-Cell Leukemia Viruses. Cold Spring Harbor Press, pp. 111-120 (1984).
2. Homma, T. *et al.* Science *225*:716-718 (1984).
3. Lee, T., *et al.* Proc. Nat. Acad. Sci. *81*:3856-3860 (1984).
4. Lee, T., *et al.* Science *226*:57-61 (1984).

1304 THE EPIDEMIOLOGY OF LEUKEMIA AND LYMPHOMA: LABORATORY IMPLICATIONS, Mark H. Greene, Environmental Epidemiology Branch, National Cancer Institute, Bethesda, MD 20205.

The leukemias and malignant lymphomas are relatively rare cancers, comprising as they do roughly 2.8% and 3.6% of all newly-diagnosed malignancies in the United States each year. Despite their rarity, these cancers have attracted a great deal of interest from the oncology research community, partly owing to advances in treatment which have rendered acute lymphocytic leukemia, Hodgkin's disease and high-grade malignant (non-Hodgkin's) lymphomas among the more treatable human neoplasias. Etiologic research has been facilitated by the development of detailed histologic classification schemes which have permitted the recognition of over a dozen specific disease entities within the overall rubric of lymphoid/hematopoietic cancers. In addition, these cancers have proven particularly amenable to laboratory-based investigations designed to clarify their etiology and pathogenesis. Rather than address the the descriptive epidemiology and risk factor profiles for each of these many different diseases, this presentation will consider selected illustrative examples of the interplay between epidemiologic/etiologic research and laboratory-based investigations, to provide models of the biochemical epidemiology research strategy and suggestions regarding future studies of this type. Thus, for example, recent cytogenetic studies employing high resolution banding techniques have revealed chromosomal abnormalities in most of the leukemia and lymphoma tumor samples tested. Further, there is evidence that many specific disease entities are associated with very specific cytogenetic abnormalities, providing evidence for etiologic heterogeneity among the diverse leukemia and lymphoma subtypes. Chronic myelogenous leukemia and Burkitt's lymphoma provide models for the nexus between specific cytogenetic abnormalties and oncogene metabolism; each has specific chromosomal translocations which result in altered oncogene expression (c-abl and c-myc, respectively). The descriptive epidemiology of Hodgkins disease has suggested that the young onset form of this cancer may have an infectious etiology The data are consistent with the hypothesis that late age-at-exposure to a common (? viral) agent is associated with an increased risk of Hodgkin's disease. Studies of EBV serology and HLA phenotype (the latter of particular interest in familial Hodgkin's disease) have provided further support for this hypothesis. Epidemiologic studies have documented the high risk of non-Hodgkin's lymphoma in patients receiving immunosuppressive medications. Molecular biologic approaches have clarified the evolution and clonality of these tumors, as well as highlighting the contribution of EBV to their development. Finally, epidemiologic surveys have documented that alkylating agents are potent leukemogens in man. These studies have shown a positive correlation between alkylating agent dose and risk of acute nonlymphocytic leukemia, and suggested variations in host susceptibility to these effects. Monoclonal antibodies raised against alkylating agent-DNA adducts may clarify how these agents induce ANL.

1305 DNA METHYLATION. Peter A. Jones, Timothy Kautiainen, Lois Chandler and Vincent Wilson. University of Southern California School of Medicine, 2025 Zonal Avenue, Los Angeles, CA 90033 and Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, MD 20205.

The methylation of cytosine residues in DNA is thought to be part of a potent gene silencing mechanism in eukaryotic cells. Undermethylation of specific sites within genes may be a necessary but not sufficient condition for their expression so that methylation plays an important role in cell differentiation. If this information coding system controls normal cell behaviour, then it follows that aberrations within it could be responsible for the aberrant gene control seen during transformation.

We have therefore investigated aspects of DNA methylation and its relationship to tumorigenesis in several experimental systems. Chemical carcinogens were found to be strong inhibitors of the in vitro catalyzed transfer of methyl groups from S-adenosylmethionine to acceptor hemimethylated DNA. The inhibitory effects of carcinogens on DNA methylation in living cells were also studied, and it was found that precarcinogens induced transient inhibition of methylation of DNA synthesized in Balb 3T3 cells. The overall methylation levels and patterns of 5-methylcytosine at the level of specific genes were investigated in cell lines and in primary explants of pediatric and adult tumors. These studies all showed aberrations in methylation control. This suggests that the generation of abnormal DNA methylation levels and patterns may play a role in the genesis of certain types of cancer. The levels of DNA methyltransferase enzyme in non-oncogenic cells and transformed derivatives from the same species were assayed. In many cases, the level of extractable DNA methyltransferase was found to be much higher in tumorigenic vs. non-tumorigenic cells suggesting that alterations in the methylating capacity of cells might accompany transformation. These findings will be discussed in relationship to the hypothesis that aberrations in methylation patterns and control mechanisms result in altered gene expression in cellular transformation.