TUMOR SUPPRESSOR GENES

Organizers: Curtis Harris and Stephen Friend

February 13 - 20, 1994; Taos, New Mexico

Plenary Sessions *Page*

Poster Sessions

Signalling Pathways

N 001 TOWARDS A GENETIC ANALYSIS OF NEUROFIBROMATOSIS TYPE 1 GENE FUNCTION. Andre Bernards, Inge The, Greg Hannigan, Anita Murthy, James Gusella, and Iswar Hariharan. Massachusetts General Hospital Cancer Center and Neurogenetics Laboratory, 149 13th Street, The Charlestown Navy Yard, Boston, MA 02129.

Neurofibromatosis type 1 (NF1), or von Recklinghausen NF is one of the most common genetic disorders of man, with an incidence of approximately 1 in 3500 births. Hallmarks of NF1 are a high mutation rate, with up to 50% of cases representing new mutations, and a high degree of variability of its symptoms. Among the most consistent symptoms of NF1 are multiple café-au-lait skin discolorations, Lisch nodules (iris hamartomas), and benign cutaneous or subcutaneous neurofibromas. In 5-10% of patients, the latter progress to malignant peripheral nerve sheath tumors, which are generally fatal. NF1 patients also show an increased frequency of various other nervous system and non-nervous system tumors. The gene responsible for NF1 was identified in 1990 and shown to encode a 2818 amino acid protein related to the *Sacharomyces cerevisiae* IRA1 and IRA2 proteins, as well as to mammalian p120GAP. This implicated the NF1 protein (neurofibromin) as a negative regulator of p21*ras*. Indeed, the GAP-related domain (GRD) of neurofibromin has been shown to bind p21*ras* up to 300 times more avidly than p120GAP itself, and to stimulate its GTPase activity. In addition, NF1 deficient tumor cells derived from NF1 patients have been found to exhibit high levels (up to 50%) of active, non-mutant *ras*.GTP. We have found that NF1 mutations, including homozygous deletions, are also frequent in cell lines derived from neuroblastoma, a neural crest derived tumor that is not associated with NF1. By contrast to the NF1 patient derived tumor cells, the NF1 deficient neuroblastomas show little, if any, increase in *ras*.GTP, suggesting that loss of another function of the NF1 protein may be more important in neuroblastoma. To allow a genetic study of NF1 function, we have cloned a closely related NF1 gene homolog from the fruit fly, *Drosophila melanogaster*. The fly homolog consists of 18 exons and spans just over 13 kb. The Drosophila gene predicts a 2764 amino acid protein that shows >60% sequence identity with human NF1 over its entire length. We have mapped the fly gene to cytogenetic interval 96F on the third chromosome, and further localized the gene to a 50 kb segment in between the *bride-of-sevenless* and *Enhancer of split* loci. We have used a fly strain with a ~200 kb deletion in this region to generate about 60 new, EMS-induced lethal mutations. These mutations fall in five complementation groups, one of which maps to a 70 kb segment around the NF1 gene. Recent results obtained with these new mutants will be presented.

N 002 MAPPING FUNCTIONAL INTERACTIONS BETWEEN RB, E2F, AND THE HPV16-E7 PROTEIN. Pearl Huang, Hans Huber, Denis Patrick, Paula Goodhart, Gynneth Edwards, Lynnette Miles, Ron Wegryzn, Mona Ivey-Hoyle, Bob Conroy, Steve Stirdivant, Stan Barnett, Lenora Davis, Vic Garsky, Allen Oliff, David Heimbrock. Departments of Cancer Research and Medicinal Chemistry. Merck Research Labs. West Point, PA 19486.

The retinoblastoma protein (pRB) is a tumor suppressor. While several proteins have been shown to bind to pRB, to date, the best understood function of the retinoblastoma protein is binding to and repressing the activity of the transcription factor, E2f. E2f is a family of at least 3 cloned genes which bind to DNA as heterodimers. That pRB/E2f binding is critical for tumor suppression is suggested by several observations: over-expression of E2f is mitogenic, over-expression of pRB represses E2f mediated transcription, and proteins which block pRB-E2f binding are oncogenic. Transformation of eukaryotic cells by the oncogenic HPV viruses has been shown to require the E7 gene, which binds to pRB. Using recombinant E7, pRB, and peptides derived from these proteins, we were able to identify two distinct domains of the E7 oncoprotein which are required to effectively compete with E2f for binding to pRB. Both of these regions, the CR2 and CR3 domains, are required for transformation. An understanding of how pRB binding represses transcription from E2f driven promoters is essential to delineate the mechanisms by which pRB helps regulate progression through the cell cycle. The effect of pRB on the E2f-DNA complex was analyzed. Even though pRB has non-specific DNA binding activity, it does not appear to make additional contacts with DNA while bound to E2f, as asssayed by DNA footprint analysis. However, E2f bends DNA upon binding to its specific site, and pRB partially reverses the effect. We propose that pRB's alteration of the E2f-induced DNA bending angle may play a role in pRB's repression of transcription from promoters dependent on E2f.

N 003 FUNCTION OF THE RETINOBLASTOMA PROTEIN, Wen-Hwa Lee and Eva Lee Center for Molecular Medicine/Institute of Biotechnology, University of Texas Health Science Center, San Antonio, Texas , 78245

To understand the biological function of RB, we have established mice model to address the role of RB during the developmental process. Our results suggest that mice without normal RB protein expression are embryonic lethal probably due to the ectopic mitosis following cell death in neuronal or hematopoetic systems. With only one copy of the RB gene, mice appear to be phenotypically normal. However, pituitary tumor originated from the intermediate lobe appears in nearly 100% of mice at the later age of about 10 months. These tumors secrete melanin stimulating hormone causing the elevation of MSH concentration in blood circulation. On the other hand, overexpression of RB protein through its own promoter resulted in dwarfism of mice. The degree of dwarfism was inversely proportional to the amount of transgenic RB protein expressed. Transferring the human RB minigene to the RB-deficient mice by crossing with the RB transgenic mice rescued their lethal phenotype. The success of the rescue depends on the amount of human RB protein expressed in the RB-deficient mice. Expression of human RB protein below the half of the wild type amount failed to rescue the mice. Deletion of the human transgene in rescued mice resulted in pituitary tumor with 90% penetrance. On the contrary to the heterozygous knock-out mice, the rescued mice are sterile, indicating the human RB transgene fails to substitute the whole mouse RB gene.

The importance of RB in human oncogenesis and mice development is evident. However, the biochemical function of RB remains to be elucidated. RB protein has an intrinsic property of forming multimer which was regulated by phosphorylation. This study provided a novel concept how RB regulates other proteins in a coordinate manner. Indeed, many cellular proteins involving in transcriptional, mitotic and enzymatic processes that bind to RB protein have been identified and thereby may mediate its function. At the regulation of G1/S transient, RB binds to E2F-1 which is responsible for transactivating several genes whose function are evident during the G1/S transition. ARP, a novel transactivator may also work at this time window. During the mitotic process, two novel proteins, mitosin and H-nuc are associated with RB, indicating a potential role of RB in the exist of mitosis. In sum, RB forms many complexes with different proteins either through sequestering and then inactivating their function, or collaborating and gaining additional positive function. Characterization of this group of proteins and their interaction mechanism should shed light on what is the role of RB in the cell.

Signalling Pathways in Lower Organisms

N 004 DIFFERENTIATION VERSUS PROLIFERATION IN THE CONTROL OF *C. ELEGANS* GERM CELL DEVELOPMENT. Ross Francis, Allan Jones, Laura Wilson and Tim Schedl, Department of Genetics, Washington University School of Medicine, St. Louis, MO.

We are using *C. elegans* as a genetic system to understand the relationship between germ cell proliferation and differentiation. Germline development requires the control and coordination of a number of processes including stem cell proliferation, entry of germ cells into meiotic prophase, progression through meiotic prophase, determination of sexual fate, and differentiation of germ cells as either sperm or oocytes. Germ cell proliferation is controlled by the distal tip cell (DTC) of the somatic gonad, which is thought to signal the germ line, promoting stem cell proliferation and/or inhibiting entry into meiotic prophase. Ablation of the DTC results in all germ cells entering meiotic prophase. The product of the *glp-1* gene is thought to be the receptor for the somatic DTC signal. Loss-of-function mutations in *glp-1* produce a germline phenotype similar to DTC ablation. *glp-1* encodes a transmembrane protein structurally related to the *Notch* family of receptors, which include the mouse *int-3* and human *TAN-1* proto-oncogenes.

We have identified mutations in two *C. elegans* developmental control genes that produce germline tumors: a dominant gain-of-function (*gf*) mutation in *glp-1* and recessive null mutations in *gld-1*. Thus, *glp-1(gf)* is analogous to activation of a proto-oncogene, and *gld-1(Null)* is analogous to inactivation of a tumor suppressor gene. Tumor formation in both mutants is multifocal. Germ cells proliferate aggressively, but are not malignant. The cellular basis of tumorigenesis in *glp-1(gf)* and *gld-1(Null)* mutants is distinct; *glp-1(gf)* germ cells fail to exit the mitotic cycle and enter meiotic prophase, while *gld-1(Null)* female germ cells fail to progress though meiotic prophase and then re-enter the mitotic cycle. Our analysis also indicates that cell-type specific developmental programs determine whether the *glp-1(gf)* and *gld-1(Null)* mutations result in a tumorous phenotype.

glp-1(gf) mutants have a temperature sensitive germline tumorous phenotype in both hermaphrodites and males. Genetic tests and laser ablation of the DTC in *glp-1(gf)* mutants indicate that the *glp-1* receptor is hyperactive and acts independent of the ligand. *glp-1(gf)* mutants also have a somatic multivulval phenotype which is the result of inappropriate specification of vulval precursor cell (VPC) fate. Thus, activation of the *glp-1* receptor causes inappropriate specification of developmental fate in one cell type (VPCs), while it causes a tumorous phenotype in another cell type (germ line). The molecular basis of *glp-1(gf)* is the replacement of a conserved Ser residue located in the extracellular domain near the transmembrane domain by Asn.

gld-1(Null) mutants have a tumorous germline phenotype in hermaphrodites while males are unaffected. Germ cell sexual fate determines whether a tumorous phenotype develops; sex determination gene mutations that masculinize the germline of *gld-1(Null)* hermaphrodites suppress the tumorous phenotype, while sex determination gene mutations that feminize the male germline induce the tumorous phenotype in otherwise unaffected *gld-1(Null)* males. We propose that *gld-1*(+) is necessary for progression through female meiotic prophase and oocyte differentiation. In *gld-1(Null)*, female germ cells enter but fail to progress through meiotic prophase and instead re-enter the mitotic cycle. The *gld-1* protein has sequence similarity to GRP33 from shrimp and GAP-associated p62 from humans in a region thought to be involved in RNA binding.

N 005 **NEGATIVE REGULATION OF A *C. elegans* TYROSINE KINASE-RAS MEDIATED SIGNAL TRANSDUCTION PATHWAY**, Paul W. Sternberg[1], Junho Lee[1], Charles Yoon[1], Gregg D. Jongeward[2], Linda S. Huang[1], Wendy S. Katz[1], & Giovanni Lesa[1],[1]Howard Hughes Medical Institute and Division of Biology, Caltech, Pasadena, CA 91125, [2]Department of Microbiology & Immunology, UCSF.

During *C. elegans* vulval induction, the anchor cell induces three epidermal vulval precursor cells to proliferate two additional rounds of mitosis and generate vulval cells. We have studied several genes required for this intercellular signaling process. *lin-3* encodes an EGF-like growth factor that is the inductive signal. *let-23* encodes an EGF-receptor-like transmembrane tyrosine kinase and is an excellent candidate for a *lin-3* receptor. *let-60* encodes a ras protein that acts like a switch whose activity state is set by inductive signal acting via *let-23*. *lin-45* encodes a homolog of the *raf* proto-oncogene, a serine/threonine kinase. *lin-45* is required for the excessive vulval differentiation caused by a constitutively activated *let-60* ras mutant, suggesting that LIN-45 acts with or after LET-60. Hyperactivity of *lin-3*, *let-23* or *let-60 ras* leads to excessive vulval differentiation. Vulval differentiation is thus analogous to cell transformation in mammalian cells. Negative regulators of vulval differentiation might then be analogous to tumor suppressor genes. We have characterized a number of genes that negatively regulate this inductive signaling pathway during vulval induction. In mutants defective in negative regulation, more than three precursor cells generate vulval cells. The *lin-15* locus encodes two novel products, LIN-15A and LIN-15B, that act upstream of LET-23 to prevent vulval differentiation in the absence of inductive signal. Both LIN-15A and LIN-15B must be inactivated to obtain excessive vulval differentiation. The *unc-101*, *sli-1*, and *rok-1* genes encode partially redundant negative regulators of *let-23* identifed by genetics screens. Mutation of only one of these three negative regulators has no effect on vulval differentiation. However, if two of these genes are inactivated, then excessive vulval differentiation occurs. Inactivation of all three genes casues an even a greater extent of vulval differentiation. *unc-101* encodes a homolog of the mammalian AP47 protein, the medium chain of the *trans*-Golgi associated "adapter protein" proposed to couple clathrin to transmembrane proteins. *sli-1* encodes a product with similarity to a mammalian proto-oncogene not previously linked with a tyrosine kinase-ras mediated signaling pathway. We are carrying out additional genetic screens to find other genes that act with *unc-101*, *sli-1*, and *rok-1* to negatively regulate vulval differentiation.

Viral and Cellular Control of Cell Cycle and Replication (Joint)

N 006 p16^{INK4}; AN SPECIFIC INHIBITOR OF THE CDK4/CYCLIN D KINASE, David Beach, Manuel Serrano and Gregory J. Hannon. Howard Hughes Medical Insitute, Cold Spring Harbor Laboratory, NY 11724.

Passage through the cell cycle in mammalian cells requires a group of related kinases known as cyclin-dependent kinases or CDKs whose activity and substrate specificity depends on their association with a family of positive regulatory subunits known as cyclins. The complexes formed by CDK4 and the D-type cyclins have been implicated in the control of cell proliferation during the G1 phase of the cell cycle. In normal, proliferating, cells, CDK4 associates with D-type cyclins and with a protein of 16 KD molecular weight, p16^{INK4} (inhibitor of CDK4). In human cells transformed with viral oncoproteins that inactivate the Rb tumor suppressor protein, p16^{INK4} is overexpressed being the main, if not exclusive, partner of CDK4. We have isolated a human cDNA clone encoding p16^{INK4} by using the two-hybrid screening system in yeast with CDK4 as the target protein. We have found that p16^{INK4} specifically associates with CDK4 *in vitro* and *in vivo*, and does not associate with other CDKs. We have used extracts from insect cells overexpressing CDK4 and D-type cyclins to reconstitute active CDK4 kinase; when these extracts are incubated with p16^{INK4} the kinase activity of CDK4 toward Rb is completely inhibited. Inhibition only occurs when using CDK4, and not CDK2, as catalytic subunit. In normal cells the balance between p16^{INK4} and D-type cyclins could determine the level of CDK4 kinase activity. Inactivation of Rb during the mid-G1 phase is thought to be fulfilled by CDK4 associated with D-type cyclins. In transformed cells expressing viral oncoproteins, such as T-antigen, E1A or E7, Rb is constitutively inactivated and the G1/S control is, at least, partially disrupted. We propose that the disruption of the G1/S control is sensed by a signalling pathway that upregulates the expression of p16^{INK4}, therefore inhibiting the CDK4 activity. In the particular case of cells expressing viral oncoproteins, this negative loop is unable to restore the Rb-dependent G1/S control because the Rb function in these cells is constitutively abrogated.

N 007 REGULATION OF THE STRUCTURE AND FUNCTION OF WILD-TYPE AND MUTANT FORMS OF p53. Jill Bargonetti, Xinbin Chen, George Farmer, Philip Friedlander, Lata Jayaraman, Jim Manfredi, Scott Miller, Yan Wang and Carol Prives. Department of Biological Sciences, Columbia University, N.Y., N.Y. 10027

The p53 tumor suppressor protein is a DNA binding transcriptional activator. Levels of wild-type p53 increase in cells after DNA damage and, depending on cell type or experimental conditions, this leads to either cell cycle arrest in G1 or apoptosis. It is likely that the ability of wild-type p53 to activate transcription is intrinsic to its ability to arrest cells. We have characterized the role of p53 as a transcriptional activator with respect to its interactions with the basal transcription factor TFIID (TBP). Our data indicate that TBP/TFIID and p53 cooperate to bind to DNA and activate transcription *in vivo* and *in vitro*. We are interested in the structure and function of wild type and mutant forms of p53. We have analyzed the domains of p53 that are involved in its DNA binding properties. Our data indicate that the central conserved region where the tumor derived mutations are located contains the sequence specific DNA binding domain. The C-terminal portion of the protein contains a region that binds non-specifically to DNA and is capable of reannealling complementary single strands of DNA. Additionally, the N-terminal and C-terminal regions of p53 contain sequences as well as phosphorylation sites that regulate the DNA binding properties of the protein. We have observed that the majority of tumor-derived p53 mutants are conditionally defective for DNA binding and display DNA binding at temperatures lower than 37° C. Therefore identifying means to stabilize the binding of mutant p53 proteins at 37° C is an important goal with therapeutic implications.

Transcriptional Control and Development

N 008 EXAMINATION OF TUMOR SUPPRESSOR GENE FUNCTION USING GENE TARGETING IN THE MOUSE, Bart Williams, Scott Lowe, Earlene Schmitt, Lee Remington, Shane Shih and Tyler Jacks, Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139 USA

Using the technique of gene targeting in murine ES cells, we have generated mouse strains carrying mutations in the tumor suppressor genes *Rb*, *p53*, *Nf1*, *Apc*, and *Dcc*. These animals were initially created as potential models for human familial cancer syndromes caused by inherited mutations of these genes. They have also allowed us to determine the developmental requirements for tumor suppressor gene function and have proven a valuable source of primary cells and cell lines with which to study gene function in vitro.

All of our strains have been extensively studied for predisposition to cancer. As reported previously, *Rb* heterozygotes do not develop retinoblastoma, but they are highly predisposed to tumors of the intermediate lobe of the pituitary. These animals also develop medullary carcinoma of the thyroid at lower frequency. In both cases, the majority of tumors show loss of the wild-type *Rb* allele. Consistent with the work of others, our *p53* mutant strain is also highly cancer prone. Heterozygous animals develop a spectrum of tumors reminiscent of that seen in humans with Li-Fraumeni syndrome, while homozygous mutant mice typically develop T cell lymphoma. In order to examine possible synergy between different tumor suppressor gene mutations, we have intercrossed the *p53* and *Rb* mutant animals and have begun to analyze the phenotype of the double mutants. The effects of heterozygosity for loss-of-function mutations in *Nf1* and *Apc* are minimal, although these animals appear to have a slightly increased cancer risk. Given that a different mutation in *Apc* causes multiple intestinal neoplasia (*min*), the weak phenotype of our *Apc* mutant strain suggests allele-specific effects.

In addition to examining the consequences of tumor suppressor gene deficiency in vivo, we have been using cells derived from homozygous mutant animals to study the function of the different genes in vitro. For example, primary cells isolated from *p53* homozygotes and controls have been analyzed for a variety of growth properties and responsiveness to a number of stimuli. These data indicate that *p53* function is critical for the normal cellular response to irradiation and exposure to certain chemotheraputic drugs. Furthermore, we have shown that, depending on the cellular environment, *p53* can induce either growth arrest or apoptosis. These results help may help explain the frequent mutation of *p53* in a wide range of tumor types. Also, the data indicate that *p53* status may be an important determinant of the efficacy of cancer therapy.

N 009 *Abstract Withdrawn*

N 010 p53 FUNCTION AND DYSFUNCTION, Jennifer Pietenpol and Bert Vogelstein. The Johns Hopkins Oncology Center, 424 North Bond Street, Baltimore, MD 21231.

Biochemical studies have suggested several potential mechanisms underlying p53-mediated growth suppression. In particular, wild-type p53 has been demonstrated to bind DNA in a sequence specific manner and to activate transcription from minimal promoters containing p53 consensus binding motifs. On the other hand, p53 has been shown to repress a wide variety of cellular and viral promoters. This transcriptional repression is unlikely to involve direct binding of p53 to promoter sequences and may be due to p53 sequestering essential transcription factors. Employing a panel of p53-derived proteins, we have analyzed the relationship between sequence-specific transcriptional activation, non-specific transcriptional repression, and suppression of tumor cell growth. Naturally occurring and in vitro-derived p53 mutants that were unable to activate transcription in a sequence-specific manner uniformly lost the ability to suppress growth but not necessarily the ability to repress transcription non-specifically. We have also defined the functional domains of p53 required for these properties. The sequence-specific transcriptional activity of p53 appeared to be essential for the protein to function as a tumor suppressor. Identification of the target genes induced by p53 expression which mediate tumor suppression is critical for unraveling the p53 pathway and is currently underway.

Control of Cellular Homeostasis

N 011 THE MERLIN TUMOR SUPPRESSOR IN NEUROFIBROMATOSIS 2, Lee B.Jacoby, Mia MacCollin, David N.Louis, James A. Trofatter, Nikolai Kley*, Bernd Seizinger*, Vijaya Ramesh, and James F. Gusella, Massachusetts General Hospital, Charlestown, MA02129, *Bristol Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543.

Neurofibromatosis 2 (NF2) is a severe autosomal dominant disorder characterized by predisposition to a number of different tumors of the nervous system, particularly vestibular and spinal schwannomas and meningiomas. These tumors also occur sporadically at a higher frequency in the general population. Genetic linkage analysis and investigation of both sporadic and familial tumors with polymorphic DNA markers have shown that NF2 is caused by inactivation of a tumor suppressor gene in chromosome 22q12. The gene encoding the NF2 tumor suppressor has been cloned recently. The gene product named 'MERLIN', encodes a 595 amino acid protein that shows striking similarity to the moesin-ezrin-radixin family of cytoskeleton associated proteins. The NF2 gene encodes atleast 17 exons with exon 16 alternatively spliced in both human and mouse. The alternatively spliced protein product encodes a 590 amino acid protein product with modified C-terminus. We have developed PCR assay for all 17 exons employing primers in the flanking intronic sequences. Using single strand conformation analysis (SSCP) as the method to scan for mutations in the NF2 gene, we have begun a detailed analysis of mutations in this gene in both NF2 related tumors such as vestibular schwannomas, meningiomas, and ependymomas as well as in non-NF2 related tumors including astrocytomas, colon cancers, melanomas, breast tumors, and neuroblastomas to understand the role of the NF2 gene as a tumor suppressor. The mutation analyses from all these tumors will be discussed.

N 012 THE TGFß FAMILY, Harold L. Moses, Mark Alexandrow, Lina Dagnino, Donald F. Pierce, Jr., and Rosa Serra, Department of Cell Biolo gy and Cancer Center, Vanderbilt University School of Medicine, Nashville, TN 37232

The transforming growth factor ßs (TGFßs), are potent inhibitors of cell proliferation and are usually secreted in a latent form. TGFß1, TGFß2, and TGFß3 are expressed in distinct but overlapping patterns in most tissues, including the developing mouse mammary gland. To study the role of transforming growth factor ß1 (TGFß1) in normal mammary development and in mammary neoplasia, we have constructed three transgenic mouse lines that express a simian TGFß1$^{S223/225}$ mutated to produce a constitutively active product under the control of the MMTV enhancer/promoter. Expression of the transgene, as confirmed by *in situ* hybridization, immunohistochemistry, RNase protection and Northern blot analysis, was associated with marked suppression of the normal pattern of mammary ductal tree development in female transgenics. Reduction in total ductal tree volume was observed at seven weeks soon after estrous begins and was most apparent at 13 weeks, as ductal growth in the normal mammary gland declines. This effect was seen in all three lines. However, during pregnancy, alveolar outgrowths developed from the hypoplastic ductal tree, and lactation occurred so that transgenic females could feed full litters. Unlike many other transgenic mouse models in which expression of growth factors, including TGF or oncogenes under control of the MMTV promoter leads to mammary epithelial hyperplasia and increased tumor formation, the MMTV-TGFß1 transgene causes conditional hypoplasia of the mammary ductal tree and no spontaneous tumors have been detected in the MMTV-TGFß1 transgenic animals. Preliminary studies involving crossbreeding of MMTV-TGFα and MMTV-TGFß1 transgenic mice indicate that expression of the TGFß1 transgene prevents the increased carcinoma development caused by expression of the TGFα transgene indicating that TGFß agonists may be useful in breast cancer prevention.

The mechanisms of TGFß1 growth inhibition have been investigated. In skin keratinocytes, TGFß1 rapidly suppresses c-*myc* expression at the level of transcriptional initiation and expression of c-*myc* was shown to be necessary for proliferation of these cells. Overexpression of c-*myc* using an inducible construct blocks growth inhibition by TGFß1. In 11.5 day p.c. lung bud organ cultures, TGFß1 inhibits tracheobronchial epithelial development including branching morphogenesis. The tracheobronchial epithelia express N-*myc* but not c-*myc* at this stage of development. TGFß1 was shown to markedly inhibit N-*myc* expression in epithelia of the lung bud organ cultures and N-*myc* gene knockout experiments by others have shown that N-*myc* is required for branching morphogenesis of the tracheobronchial tree as well as other epithelial structures. The data indicate that the TGFßs are important negative autocrine growth regulators *in vivo* and suggest that suppression of expression of either N-*myc* or c-*myc* depending on the cell type involved may play a role in TGFß growth inhibition.

Genetic Instability I (Joint)

N 013 P53 AND OTHER MOLECULAR CONTROLS OF THE RESPONSE TO DNA DAMAGE, Michael B. Kastan, William G. Nelson, Chaw-Yuan Chen, and William B. Slichenmyer, The Johns Hopkins Oncology Center, Baltimore, Md. 21287.

A variety of cellular responses to DNA damage influence cellular fate, such as whether heritable genetic alterations are passed on to daughter cells and whether the cell survives the damaging insult. Efficiency of repair of the damage and the timing of this repair relative to critical cellular processes, such as DNA replication or chromosome segregation, are two important parameters dictating cellular outcome. We have recently characterized a signal transduction pathway which dictates whether or not a cell will arrest in G1 following exposure of mammalian cells to various DNA damaging agents. P53, the most commonly mutated gene in human cancers characterized to date, is a critical participant in this cellular response pathway. Levels of p53 protein transiently increase by a post-transcriptional mechanism in response to DNA damage and result in a transient G1 arrest. Cells lacking normal p53 function continue to enter S-phase and replicate their DNA despite the presence of the DNA damage. The gene product(s) which is(are) defective in the cancer-prone disease, ataxia-telangiectasia (A-T), is required for optimal induction of p53 protein by ionizing radiation since cells from A-T patients are quantitatively defective in p53 induction. DNA damage-induced increases in p53 protein also result in increases in transcription of the GADD45 and MDM2 genes; the former gene product may be involved in mediating the G1 arrest and the latter may participate in a "feedback" loop which limits the length of the G1 arrest. Though exposure of cells to many different types of DNA damaging agents results in p53 induction, it appears that DNA strand breaks are sufficient and are probably a necessary intermediate signal in the DNA for initiation of this pathway. In some cell types, initiation of this pathway results in apoptotic cell death rather than in a G1 arrest; in these cells, loss of p53 function results in a relative increase in resistance to the cytotoxic effects of the DNA damage. Interestingly, however, in contrast to the consequences of loss of the G2 checkpoint in yeast when the rad9 gene is mutated, in mammalian cells in which a G1 arrest (rather than apoptosis) is the usual result of the DNA damage, no change in radiosensitivity is noted when p53 function is disrupted. Further characterization of the molecular steps in this pathway and the physiologic ramifications of dysfunction of the pathway is continuing.

N 014 ABROGATION OF CELL CYCLE CHECKPOINT CONTROL IN PRENEOPLASTIC CELLS, Thea D. Tlsty*†, *Lineberger Comprehensive Cancer Center, Department of Pathology, and †Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC 27599-7295

Genomic integrity is maintained by a network of cellular activities that assesses the status of the genome at a given point in time and provides signals to proceed with or halt cell cycle progression. Mutations in any part of these cellular pathways can have the ultimate effect of disrupting chromosomal integrity. We have used viral proteins involved in malignant transformation to investigate cellular pathways that may be perturbed during loss of genomic stability. Recent studies have identified cellular proteins which are targets for the viral oncoproteins, stressing the importance of these cellular proteins in controlling neoplasia. Among the targets of the viral oncoproteins are the products of the p53 and retinoblastoma (Rb) tumor suppressor genes. We demonstrate that the expression of human papillomavirus type16 E6 and E7 oncoproteins in normal, mortal cells disrupts the integration of the network of signals that maintain genomic integrity. E6-expressing cells, in which cellular p53 protein is bound and degraded, exhibited alterations in cell cycle control and displayed the ability to amplify the endogenous CAD gene when placed in the drug PALA. Expression of E7, which complexes with a variety of cellular proteins including Rb, resulted in an p53-independent alteration in cell cycle control, massive cell death, and polyploidy upon PALA treatment. These results demonstrate that the viral proteins disrupt cellular processes that safeguard the genome and growth of normal cells. Alterations of these controls are being examined in cells from patients that are predisposed to neoplasia.

Genetic Instability II

N 015 G2 CONTROL IN MAMMALIAN CELLS: IDENTIFICATION OF NOVEL MITOTIC INHIBITOR, Hiroto Okayama[1,2], Makoto Igarashi[2], Akihisa Nagata[1], and Shigeki Jinno[1], [1]Department of Biochemistry, Faculty of Medicine, The University of Tokyo, Tokyo, Japan and [2]The Okayama Cell Switching Project, ERATO, JRDC, Kyoto, Japan

In mammalian cells, cdc2 kinase plays a key role in the initiation of mitosis. During the S and G2 phases, the kinase becomes phosphorylated at Thr161, Thr14 and Tyr15, and is held inactive. At the end of the G2 phase, it undergoes rapid dephosphorylation at Thr14 and Tyr15, and concomitant activation, resulting in triggering the initiation of mitosis. Wee1 kinase phosphorylates Tyr15 and inhibits mitosis whereas cdc25B and cdc25C dephosphorylate both residues and induces mitosis. Such a mitotic control involving wee1 and cdc25 as key regulators is evolutionarily conserved from yeast through mammals.

Using trans-complementation of a fission yeast mutant, we have cloned a human cDNA(**min1**) encoding a novel mitotic inhibitor. The **min1** cDNA effectively rescues Δmik1 wee1-50 and wee1-50 cdc2-3w, indicating that it is an inhibitor of cdc2 kinase. Consistent with the complementation data, the **min1** protein appears to inhibit cdc2 kinase in an *in vitro* assay. A 129 kDa full length **min1** protein has only a residual activity, but is markedly activated by truncation at the N-terminal region, suggesting that the activity of the **min1** protein itself is regulated. Like cdc2 and wee1, **min1** is predominantly expressed in G2. A nonsense mutation is found in one allele of this gene in an SV40-transformed human cell line. Thus, a novel mitotic regulatory system seems to exist in mammalian cells, and its mutation may promote genetic instability. In addition, we have isolated a human cDNA encoding a protein kinase that suppresses Δmik1 wee1-50. Characterization of this gene is in progress.

N 016 TARGETS OF p53. Arie Zauberman, Ayelet Lupo, Tamar Juven, Rebecca Haffner, Eyal Gottlieb, Yaacov Barak, and Moshe Oren, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Potential targets of the wt p53 protein have been looked at biochemically and biologically. At the biochemical levels, genes containing p53-responsive elements have been characterized. One such target is the LTR of a mouse endogenous retrovirus-like element (GLN element). The GLN LTR contains a high-affinity p53 binding site, which matches the El-deiry p53-binding consensus in 20/20 positions. The GLN LTR is transcriptionally activated by wt p53. Moreover, the expression of the natural, endogenous GLN elements is strongly stimulated upon induction of wt p53 activity in cells carrying the temperature-sensitive (ts) p53 mutant p53val135. Another putative p53 target is the mdm2 gene. Overexpression of mdm2 inhibits the activity of wt p53, apparently through interaction of some of the MDM2 polypeptides with the p53 protein. The mdm2 gene binds p53, and is strongly stimulated by p53 overexpression. The p53 responsive promoter is located within the first intron of the mdm2 gene. The data raise the possibility that p53 activates an internal mdm2 promoter, and alters not only the amount, but also the qualitative pattern of expression of mdm2 transcripts and probably also of MDM2 proteins. Possible implications of this prediction will be discussed. In addition, data will be presented which suggest that wt p53 may bind directly to certain immunoglobulin gene promoters. Such interaction is particularly interesting in view of the reported relationship between p53 and B cell differentiation.

At the biological level, p53 has been shown to be involved in certain types of cell death, particularly after exposure to DNA damage. Data will be presented that suggest a role for p53 also in mediating death of cells deprived of hematopoietic survival factors. Specifically, the death of IL3-dependent lines can be delayed by stably infecting them with retroviruses expressing negative dominant p53 mutants prior to IL3 withdrawal. This is consistent with the possibility that loss of p53 function may sometimes contribute to carcinogenesis by promoting illegitimate cell survival.

N 017 UBIQUITOUS SOMATIC MUTATIONS IN SIMPLE REPEATS DEFINE A NOVEL MUTATOR MECHANISM FOR ONCOGENESIS Manuel Perucho, Yurij Ionov, Miguel Angel Peinado, Sergei Malkhosyan, Antonia Velazquez, Sofia Casares, Angelina Quintero and Darryl Shibata[1]. California Institute of Biological Research. 11099 North Torrey Pines Road, La Jolla, California, 92037. [1]: Department of Pathology, University of Southern California School of Medicine, Los Angeles, California 90033.

We have recently reported that a subset of colorectal tumors accumulate clonal ubiquitous somatic mutations (USM) in simple repeated sequences (SRS) in numbers that surpass the hundreds of thousands (Ionov *et al*, Nature, *363*, 558, 1993). Because these mutations were significantly associated with distinctive genotypic (low incidence of *ras* and p53 gene mutations), phenotypic (poorly differentiated carcinomas of the proximal colon) and clinical (early cancer onset and low incidence of metastases) characteristics, we concluded that USM in SRS reveal a new molecular mechanism for colon tumorigenesis, corresponding to the "cancer as a mutator phenotype" hypothesis (Loeb, Cancer Res. *51*, 3074, 1981). Because these clonal mutations were present in all neoplastic areas of each of synchronous tumors from the same patient, including adenomas, we inferred that they are the consequence of a "*mutator mutation*" (a mutation in a gene coding for a replication or repair factor that results in decreased replication fidelity) which plays an ultimate causal role in tumorigenesis. We also suggested that USM in SRS are due to slippage by strand misaligment (Streisinger *et al*, Cold Spring Harbor Symp. quant. Biol. 31, 77, 1966) and that the mutator mutation may have a hereditary predisposition, with tumors with USM in SRS overlapping with those of the hereditary nonpolyposis colorectal cancer (HNPCC) Lynch Syndrome.

While the genomic instability in these repeated sequences is likely due to a defect in replication/repair, the gene(s) responsible for this massive accumulation of mutations and the exact timing of their occurrence are unknown. It is also not clear whether this defect in replication or repair continues during tumorigenesis or whether the mutator phenotype could be deleterious to the cancer cell and be selected against during tumor progression. We have determined the mutation rates of these sequences *in vitro* and *in vivo*. We show that USM in SRS are very early events in tumor development, and that the instability in these repeated sequences persists in malignant cells of the mutator phenotype, which continue to accumulate mutations during tumorigenesis as consecutive slippage events of a single or a few repeated units. We conclude from these results that this is the same mechanism underlying the repeat expansions of triplet hereditary diseases and that the same defect in replication fidelity observed in colon cancer of the mutator phenotype may also contribute to the non Mendelian anticipation of these diseases.

Clinical Implications

N 018 MUTANT P53 TUMOR SUPPRESSOR PROTEIN AS AN IMMUNOTHERAPEUTIC TARGET David P. Carbone[1], Michael Yanuck[2], Elizabeth Wiedenfeld[1], Frank Ciernik[1], Marcelo Fernandez-Viña[1], C. David Pendleton[2], Taku Tsukui[2], John D. Minna[1], and Jay A. Berzofsky[2]. [1]UT Southwestern Medical Center, Dallas, Texas 75235, and [2]Molecular Immunogenetics and Vaccine Research Section, Metabolism Branch, NCI, NIH, Bethesda, MD 20892.

More than half of all human lung cancers contain mutant p53 proteins, the single most common genetic lesion yet identified in human cancer. These mutant proteins are absolutely tumor specific and could potentially be used as immunotherapeutic targets. Since cytotoxic T cells (CTL) recognize intracellular and even nuclear antigens which are processed into peptides that are presented on the cell surface on class I major histocompatibility (MHC) molecules, these mutant oncoproteins do not have to be normally present on the cell surface to be recognized by CTL. We asked whether we could induce a CTL response against mutant p53 proteins epitopes by peptide immunization, and whether mutant p53 could be processed and presented to allow specific recognition and killing of cells expressing it by these CTL. Mutant p53 - specific CTL were generated by immunizing BALB/c mice with spleen cells pulsed with a peptide spanning the mutation encoded in a mutant p53 cloned from a human lung cancer. These CTL recognized peptide-sensitized targets as well as fibroblasts transfected with the intact mutant p53 open reading frame in an expression vector, but not control targets expressing a different mutant human p53. These CTL were K^d restricted and blocked by anti-CD8 monoclonal antibodies. These results show that mutant human p53 peptides can induce CTL which specifically lyse cells endogenously expressing the intact mutant p53 protein.

If this presentation is of physiological significance, one might expect that tumor cells containing mutant p53 epitopes that were efficiently presented on class I MHC would be selected against and not give rise to clinical tumors unless they had other mechanisms to escape recognition. Peptides bound to the most prevalent human class I MHC molecule, HLA-A*0201, are nonamers with conserved residues at positions 2 and 9. Therefore, missense mutations identified in tumors arising in HLA A*0201 positive patients should lie outside of this motif. The location of 299 missense mutations in exons 5 through 8 from patients of unknown HLA type were evaluated. 36/299 were within this motif, statistically fewer than would be expected if mutations were independent of location of motifs ($p < 0.001$). This model predicts that tumors that do exhibit mutations within the peptide binding motif for the HLA-A*0201 allele should not have the A*0201 allele. 6 of 50 missense mutations in lung cancer cell lines of unknown HLA type that were available for study were predicted to be presented by HLA-A*0201. By allele specific PCR and oligonucleotide hybridization, we determined that, while A*0201 has a 46% prevalence, none of these 6 tumors expressed A*0201 or closely related alleles, in accordance with the model ($p = 0.02$, binomial test). One patient had A2 in his constitutional DNA but had lost it in the tumor. This analysis suggests that human mutant oncoproteins might undergo selection for those that cannot be presented on class I MHC molecules *in vivo*. This might represent a significant selection pressure in the development of human cancer.

N 019 ALTERED PATTERNS OF TUMOR SUPPRESSOR GENES IN HUMAN TUMORS: CLINICAL IMPLICATIONS. Carlos Cordon-Cardo. Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

The etiopathogenesis of neoplastic diseases is characterized by its multiple nature. Biological, chemical, and physical agents have been identified as initiating or promoting neoplastic mechanisms. However, they all appear to a have common molecular basis, granting genetic instability and causing somatic derrangements to preneoplastic and tumor cells. In addition to these somatic mutations, which are the most frequent abnormalities identified in human cancer, the germ-line mutations associated with specific familial cancer syndromes have been recently characterized.

Human neoplasms have been classified histogenically according to their morphological resemblance to normal cognate tissues. However, as important as the histogenetic categorization provided by this classification scheme is, it addresses only indirectly other aspects of human neoplasia, which are more relevant to their behavior and the selection of therapy. These include the rate of proliferation, capacity for invasion and metastases and response to various forms of therapy. It is well known that morphologically similar tumors may behave in radically different fashions, a fact that seriously hampers the ability to accurately predict clinical outcome and properly designed therapeutic intervention in a given case.

The use of modern molecular and immunochemical techniques has led to remarkable progress in our understanding of cell growth and differentiation, these being key issues in tumor development and progression. Multiple molecular abnormalities, including activation of proto-oncogene and deactivation of tumor suppressor genes, have been identified in most human cancers studied. The accumulation rather than the order of these genetic alterations appears to be the most important event. The final result is a selective growth advantage that allows cancer cells to create an uncontrollable wide-spread disease to which patients succumb. Biologic markers that correlate with tumor behavior and response to therapy are constantly being identified. The implementation of these objective predictive assays to our armamentarium of diagnostic and prognostic tools will enhance our ability to assess tumor biological activities and to design effective treatment regimens. The need now is to translate this newly developed scientific knowledge into diagnostic and therapeutic strategies. The applicability of these techniques to clinical samples will be discussed. Using the studies on altered patterns of p53 and Rb on tumor progression in bladder cancer and other tumors, we will address the impact of these markers in the areas of tumor diagnosis and prognosis. It is our hope that the knowledge we are obtaining through immunopathology and molecular pathology of neoplastic diseases will provide some solutions for our persistent problems in the early detection, better classification and prognosis of cancer.

Late Breaking News II (Joint)

N 020 THE USE OF INTERFERONS TO RESCUE TWO NOVEL DEATH ASSOCIATED GENES AND TO IDENTIFY MOLECULAR MECHANISMS OF CELL CYCLE ARREST, Adi Kimchi[1], Dror Melamed[1], Naomi Levy[1], Lena Feinstein[1], Louis Deiss[2], Hanna Berissi[1], Tal Raveh[1], Nava Tiefenbrun[1] and Ofer Cohen[1], [1] Department of Molecular Genetics and Virology, The Weizmann Institute of Science, Rehovot 76100, [2] The George Williams Hooper Foundation,University of California, San Francisco, California.

Interferons (IFNs) and IL-6 are potent growth inhibitory cytokines that induce cell cycle arrest at the G0/G1 phase in a variety of hematopoietic and epithelial cells. The molecular mechanisms through which the cytokine-receptor interactions modify the expression or the function of few cell cycle controlling genes were analysed in order to elucidate the genetic control of proliferation arrest in mammalian cells (reviewed in 1).Two of the recently studied downstream target genes will be discussed: E2F and wt p53. We found that IFNs (α or β) or IL-6 suppressed within few hours the DNA-binding activity of E2F in hematopoietic cells. The inhibitory effect could be reversed by the removal of Mg^{2+} cations from the cell extracts suggesting that the cytokines either modified E2F post-translationally or induced/activated a putative DNA-binding inhibitor (2). This novel type of E2F regulation by IFNs and IL-6 contributed to the cytokine-induced c-*myc* suppression. Single and double transfections with wt p53 and deregulated *c-myc* proved the existence of complementary interactions between the outcome of p53 activation and of c-*myc* suppression in the control of the cytokine-triggered cell cycle arrest (3).
In some cells IFN-γ also induces programmed cell death that is characterized by distinct cytological features.We have employed a method of anti-sense selection of genes (4) to rescue cDNAs that mediate this type of cell death. The cells were transfected with an anti-sense cDNA expression library, cloned in EBV-based vector and episomal DNA was rescued from cells that survived in the presence of IFN-γ. Two novel anti-sense cDNA clones were isolated, each suppressing the occurence of the death associated morphological alterations in the continuous presence of the cytokine. One, designated DAP-1, is expressed as a single 2.4 Kb mRNA transcript which codes for a 15kDal basic protein that carries the RGD motif and few potential phosphorylation sites. The second, is transcribed into a single 6.3 Kb mRNA which has the potential to code for a unique calmodulin-dependent serine/threonine kinase (termed DAP-kinase) that carries 8 ankyrin repeats . The two genes are widely expressed in a variety of cells and may represent novel cell death associated genes.

References: 1. Kimchi,A. (1992). J. Cell. Biochem. 50:1-9. **2.** Melamed, D., Tiefenbrun, N. Yarden, A. and Kimchi, A. (1993). Mol. Cell. Biol.13, 5255-5265. **3.** Levy, N. Yonish-Rouach, E. Oren, M. and Kimchi, A.(1993) Mol. Cell. Biol. in-press. 4. Deiss, L. and Kimchi, A.(1991) Science 252, 117-120.

Signalling Pathways and Cell Cycle

N 100 REGULATION OF MYC-MEDIATED TRANSCRIPTION BY THE RETINOBLASTOMA TUMOR SUPPRESSOR GENE PRODUCT AND P107, Jalila Adnane and Paul D. Robbins, Department of Molecular Genetics and Biochemistry, Uniiversity of Pittsburgh School of Medicine, Pittsburgh, PA 15261

The c-Myc protein can function as a classical transcription factor. It is sequence-specific DNA binding protein when complexed with Max protein and contains a strong transactivation domain in its amino terminus. It has been reported that bacterially-expressed chimeric proteins containing the amino-terminus of Myc can specifically interact with Rb tumor suppressor protein *in vitro*. This suggests that Rb may directly regulate the function of Myc transcription factor through protein-protein interaction. To determine whether the observed interaction reflects a physiologically relevant association *in vivo*, we have examined the ability of Rb to regulate Myc-mediated transcription in various cell types. We have used GAL4-c-Myc fusion proteins in cotransfection assays with a GAL4-dependent reporter plasmid and a Rb expression vector to determine if Rb regulates Myc-mediated transcription independent of associations with other H-L-H proteins such as Max. Moreover, we have used a two hybrid system to examine whether Rb and Myc are able to interact *in vivo*. Our results demonstrate that Rb can modulate Myc protein activity in a cell-type dependent manner through either a direct or indirect interaction. We also have examined the ability of p107 to regulate Myc mediated transcription using similar assays. We have demonstrated that p107 is able to significantly repress Myc-mediated transcription in a transient cotransfection assay. Moreover, the two hybrid system was used to demonstrate that p107 is able to interact with Myc *in vivo*. The detailed analysis of the domain(s) in Rb and p107 as well as the domain(s) in Myc that are required for the observed interactions will be presented.

N 101 DEVELOPMENT OF AN *E. COLI* EXPRESSION AND PURIFICATION SYSTEM FOR RETINOBLASTOMA PROTEIN: IMPLICATIONS FOR GROWTH SUPPRESSION IN VITRO AND IN VIVO, Douglas E. Antelman, Bernard Huyghe, Duane Johnson and H. Michael Shepard, Canji, Inc. San Diego, CA 92130.

The loss of functional retinoblastoma (Rb) protein appears to be associated with the development of many human cancers, including breast, bladder, small-cell and non-small-cell lung carcinomas and acute myelogenous leukemia. Mutational inactivation of the Rb gene in these and other cancers results in the absence of normal transcriptional control of cell-cycle associated genes. Recent reports show that the replacement of an Rb(+) gene into a Rb(-) phenotype can partially or fully suppress the transformed phenotype. An alternate approach to gene therapy is the reintroduction of Rb protein into Rb(-) cells.

We have developed a novel E. coli expression and purification system that is capable of producing milligram quantities of p110Rb. Recombinant Rb introduced exogenously into the growth medium specifically inhibits the growth of a variety of Rb(-) but not Rb(+) tumor cell lines and is active for E2F binding in an in vitro assay. Furthermore, peritumoral or systemic injection of recombinant Rb inhibits growth of xenografted tumors in nude mice. These results indicate a potential therapeutic use of recombinant Rb. The various parameters that govern expression and recovery of Rb as well as a possible mechanism of action will be discussed.

N 102 **DIMERIZATION OF HUMAN D CYCLINS**

Steven F. Dowdy, Philip W. Hinds, Kenway Louie, Elinor Ng-Eaton, Mary W. Brooks, and Robert A. Weinberg, The Whitehead Institute for Biomedical Research, Cambridge, MA 02142

Cyclin D1 is required for cell cycle progression up to the G1/S phase transition. We have previously shown that cyclin D1 is capable of cooperating with the 928 non-pRb-binding E1A mutant to transform baby rat kidney cells (BRK). Alteration of an evolutionarily conserved lysine residue in the cyclin box domain of cyclin D1 inhibited the ability of wild type cyclin D1, D2 and D3 to transform BRK cells when cointroduced with the mutant cyclin D1 allele. This apparent dominant-negative mode of action could not be readily explained by direct nonproductive interactions with cyclin-dependent kinases (cdks). However, characterization of this mutant cyclin D1 protein resulted in the identification of cyclin D1 homo-dimers and subsequent identification of cyclin D1:D2 and D1:D3 heterodimers. Cyclin D1 homodimers appear to dissociate after interacting with cdk4, forming heterodimers with the cdk4m. These observations suggest a molecular explanation of the apparent dominant-negative properties of the mutant cyclin D1 protein as has been observed for the products of dominant-negative alleles of other genes such as p53. In addition, heterodimers of different cyclin D family members may dictate which cdk subunit can bind and therefore become activated in a tissue-specific fashion. This type of combinatorial mechanism may allow the cell a multitude of possibilities for regulating the targets of cyclin D:cdk complexes.

N 103 **OVEREXPRESSION OF THE p53 TUMOR SUPPRESSOR GENE IN MYELOID LEUKEMIC CELLS, Mats Ehinger, Inge Olsson and U Gullberg. Dept. of Hematology, University of Lund, Lund, Sweden**

Mutations of the p53 tumor suppressor gene are profoundly involved in the development of cancer. Although first characterized as a protooncogene, it was later shown that all p53 proteins with transforming properties were mutant forms. By contrast, wild type p53 protein has no transforming properties. Instead, overexpression of wild type p53 in some cell lines lacking endogenous p53 suppresses the malignant phenotype. We have decided to overexpress wild type p53 in leukemic cell lines in order to investigate the consequences for growth, differentiation and apoptosis. The human myeloid leukemic cell line K562 was transfected with the expression vector pc53SN3 coding for human wild type p53 protein. After selection, stable subclones were expanded and expression of p53 protein was characterized by biosynthetic labeling and immunoprecipitation with the monoclonal antibodies Pab421, Pab1801, DO-1 (all reacting with wild-type and mutant human p53), Pab246 (reacting with wild-type murine p53, used as a negative control), Pab1620 (reacting with wild-type human p53) and Pab240 (reacting with mutant human p53). Our results indicate that a similar pattern of immunoreactive p53-protein was present in the K562 p53-subclones. Absence of reactivity with the mutant conformation specific antibody Pab240 suggests that the expressed p53 was of wild type conformation. When assayed for proliferation in suspension culture, the growth rate of transfected cells did not differ from that of control cells. Nor was the capacity for clonal growth in semisolid medium significantly different between p53-clones and control cells. The effect of sodium butyrate, all trans retinoic acid, interferon-gamma on clonogenic growth was also similar in transfected clones and in control cells. However some of the transfected clones were more sensitive to clonal growth inhibition by TNF than control cells. This difference in sensitivity to TNF did not seem to depend on a changed sensitivity for induction of apoptosis. Our results suggest that overexpression of wild type p53 in this cell line lacking endogenous p53 does not necessarily diminish the proliferative capacity of the cells but that p53 may be involved in TNF-induced inhibition of growth. This inhibition seems to be independent of induction of apoptosis.

N 104 Characterization of growth-arrest genes induced by genotoxic stress and roles for the tumor suppressor p53 in their regulation. **Albert J. Fornace, Jr., France Carrier, M. Christine Hollander, Martin L. Smith, Dan Liebermann*, Barbara Hoffman*, and Qimin Zhan, Lab. of Molecular Pharmacology, NIH, Bethesda, MD, and *Fels Institute, Temple University School of Medicine, Phila., PA.**

In mammalian cells the G_1 checkpoint activated by ionizing radiation (IR) has recently been found to be dependent on the p53 tumor suppressor that is required for induction of the *GADD45* gene[1]. p53 protein levels, sequence-specific DNA-binding of p53, and p53 transcriptional activity are induced by IR and also by a variety of other stresses including UV radiation, alkylating agents, and medium depletion (starvation)[2]. The spectrum of inducing agents for *GADD45* and the other gadd genes is very similar to that for p53 activation. Current studies indicate that in addition to the p53 requirement for induction of *GADD45* by IR, p53 can have a cooperative effect in the induction of the gadd genes by a variety of stresses. Evidence includes reduced responses in mutant cells, such as ataxia telangiectasia, and reduced induction with gadd-promoter CAT-reporter gene constructs in cells containing dominant-negative p53 expression vectors. Interestingly, the protein products of 3 gadd genes plus a related gene *MyD118* that is also growth-arrest and DNA-damage inducible, encode unusually acidic proteins with very similar charge characteristics; both this property and a similar pattern of induction is shared with another p53-regulated gene *MDM2*. Like several other members of this group, we have recently found that Gadd45 is a nuclear protein, whose level increases after genotoxic stress. The use of a short term transfection assay, where expression vectors for one or a combination of these gadd/MyD genes were transfected with a selectable marker into several different human tumor cell lines, has provide direct evidence for the growth inhibitory functions of the products of these genes and their ability to cooperate in growth suppression. Taken together, these results indicate that these genes are down-stream effectors of p53-mediated responses involved in growth control after genotoxic stress.

[1] Kastan, M. B., Zhan, Q., El-Deiry, W. S., Carrier, F., Jacks, T., Walsh, W. V., Plunkett, B. S., Vogelstein, B., and Fornace, Jr, A. J. (1992). A mammalian cell cycle checkpoint utilizing p53 and *GADD45* is defective in ataxia telangiectasia. Cell *71*, 587-597.

[2] Zhan, Q., Carrier, F., and Fornace, Jr, A. J. (1993). Induction of cellular p53 activity by DNA-damaging agents and growth arrest. Mol. Cell. Biol. *13*, 4242-4250.

N 105 RETINOBLASTOMA PROTEIN AND CONTROL OF HUMAN LUNG CANCER CELL REPLICATION, Edward W. Gabrielson, Yuan Lin, Weibo Zhou, and Theresa Cho, Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21224

Replication of several human non-small cell lung cancer cell lines (EKVK, CaLu1, SK-MES and A549) is reversibly inhibited in the G_1 phase of cell cycle by low levels of protein kinase inhibitors such as staurosporine, chelerythrine, and H7. These agents are all non-specific, but share variable degrees of specificity for protein kinase C. Several observations suggest that the retinoblastoma gene product (pRB) is involved in this pharmacologically induced growth arrest. For example, phosphorylation of pRB was observed by Western analysis to decrease dramatically in EKVX cells within 4 hours of staurosporine treatment, preceding the accumulation of growth arrested cells in G_1. Increased pRB phosphorylation was observed within 8 hours after removal of staurosporine from the medium, which precedes the re-entry of cells into S phase. To more directly examine the role of pRB in the control of EKVX cell replication, cultures were transfected with a plasmid containing the pRB inactivating HPV oncoprotein E7. This resulted in cell cultures that are strikingly resistant to inhibition of replication by staurosporine. These data suggest that pRB is an important control of cell replication for some human lung cancers, and that a staurosporine-sensitive protein kinase may stimulate replication of these cells by phosphorylation and inactivation of pRB.

N 106 INVOLVEMENT OF p53 IN TRANSFORMING GROWTH FACTOR-ß1 INHIBITION OF GROWTH, Garcia, Anna Marie and Moses, Harold L., Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37235

Transforming Growth Factor ß1 (TGFß1) is a potent inhibitor of cell proliferation. This negative growth factor has been shown to suppress mRNA levels of c-*myc* , a cell cycle regulatory protein, in a variety of cell types at any point in G1. C-*myc* expression appears to be necessary for cell cycle progression up to the G1/S boundary. Many cancer cells lose their growth inhibitory response to TGFßs, although most still retain TGFß receptors.

p53 is the gene currently known to have the most frequent mutations in common human cancers. p53, a tumor suppressor which is thought to be potentially involved in TGFß growth inhibition, is capable of down regulating c-*myc* transcription. Mutant forms of p53 are either incapable of suppressing c-*myc* transcription, or in some cases may cause transactivation of c-*myc*.. If p53 plays a possible role in the TGFß1 pathway for growth inhibition or c-*myc* suppression, introduction of wild type p53 into TGFß1 resistant cells with double genetic p53 knockouts should restore TGFß1 sensitivity.

Mammary epithelial cells and primary keratinocytes derived from mice expressing wild type p53 are inhibited by TGFß1, both in terms of growth and radiolabeled thymadine incorporation. However, mammary epithelial cells derived from p53 homozygous null mice or containing a mutant form of p53 are resistant to growth inhibition by TGFß1, even though they express TGFß receptors.

Northern analysis of c-*myc* indicates a decrease in mRNA levels in response to TGFß1 in primary keratinocytes and mammary epithelial cells derived from normal mice, but not a significant decrease in cells lacking p53 or the mutated p53 expressing mammary epithelial cells.

Primary p53 null keratinocytes and mammary epithelial cells have been infected with wild type and several mutant forms of p53 cDNAs. An advantage of utilizing p53 null cell lines is that no interference from an endogenous p53 gene exists, and the cells do not have the numerous mutations that commonly occur in cancer cell lines. The growth characteristics, responsiveness to TGFß1 and c-*myc* expression in the above infected cells are assessed.

N 107 CELL CYCLE DEPENDENT EXPRESSION OF MDM-2 IN NORMAL AND IMMORTALIZED HUMAN BREAST EPITHELIAL CELLS, Jean M. Gudas, Hoang Nguyen and Kenneth H. Cowan, Medical Breast Cancer Section, Medicine Branch, National Cancer Institute, Bethesda, MD 20892

Mdm-2 is a putative nuclear transcription factor that is capable of interacting with and modulating the activity of the p53 tumor suppressor gene. To better understand the role of this protein in cells derived from different stages of tumor progression, we have examined the expression of mdm-2 in synchronized populations of normal (NMECs) and immortalized human mammary epithelial cells . Our results indicate that there is no correlation between mdm-2 mRNA and protein levels during the cell cycle in any of these cell types. Mdm-2 mRNA levels are increased in arrested NMECs, decreased as they progress through G1 and increased again once the cells have reached S phase. In contrast, mdm-2 protein levels are low in the arrested cells and peak during late G1 and early S phase. When two immortalized, nontumorigenic breast epithelial cells, MCF10 and 184B5, were synchronized by growth factor withdrawal, mdm-2 mRNA levels did not change significantly during the cell cycle. Mdm-2 protein levels were decreased in the arrested cells and increased again prior to entry of the cells into S phase. Further work is in progress to correlate these data with p53 expression and to identify the cellular proteins that interact with mdm-2 at different times during the cell cycle.

N 108 THE 5'-FLANKING REGION OF THE MURINE CYCLIN D2 GENE CONTAINS "E-BOX" ELEMENTS THAT BIND MYC AND MAX, Joan Hanley-Hyde and G. Jonah Rainey Lab. of Genetics, National Cancer Institute,NIH Bethesda, MD 20892

Sequencing of genomic DNA from the 5' flanking region of the murine cyclin D2 gene demonstrated the presence of two potential "E-box motifs". Electrophhoretic mobility shift assays using protein extracts from several tumors of B cell origin and end-labelled oligonucleotides containing each of these motifs showed complex-formation. The mobility of some of the complexes was super-shifted in the presence of antibody against either c-Myc or Max. Experiments to characterize these complexes further are currently underway.

N 109 **INTEGRATION OF MULTIPLE G1 CYCLIN SIGNALS IN pRB HYPERPHOSPHORYLATION,** Masanori Hatakeyama,Julie A.Brill, Gerald.R.Fink and Robert A.Weinberg, The Whitehead Institute of Biomedical Research, Cambridge, MA 02142

The retinoblastoma gene product (pRB) constrains cell growth by preventing cell cycle progression from G1 to S phases. The pRB function appears to be controlled physiologically by cell cycle-dependent hyperphosphorylation. This pRB phosphorylation can be faithfully reproduced by expressing human pRB in the yeast *Saccharomyces cerevisiae*. As is the case in mammalian cells, the phosphorylation requires an intact pRB pocket domain which is involved in pRB-target molecule interaction. Phosphopeptide mapping analysis of pRB expressed in yeast showed that the detailed biochemical nature of the hyperphopsphorylation is quite similar to that in mammalian cells. Furthermore, induction of pRB hyperphosphorylation is totally inhibited by a yeast negative growth factor, mating pheromone. Expression of pRB in non-pheromone responsive mutants as well as *CLN*(-) mutants indicated that particular combinations of the yeast G1 cyclins are required for the induction of pRB hyperphosphorylation. Moreover, mammalian G1 cyclins expressed in yeast can complement the function of yeast G1 cyclins. The results indicate that multiple G1 cyclins are involved in the phosphorylation of pRB to regulate its function.

N 110 Generation of Interleukin-3 (IL-3) autocrine mast cell tumors by v-H-ras involves transcriptional or posttranscriptional alterations in the control of IL-3 expression Hans H. Hirsch, Asha P.K. Nair, and C. Moroni, Institute for Medical Microbiology of the University of Basel, Pertersplatz 10, CH-4003 Basel, Switzerland

Activating mutations in the ras oncogene are not sufficient to induce malignant transformation, but require further cooperating alterations. Expression of the v-H-ras oncogene in the IL-3 dependent PB-3c mast cells leads to the generation of two classes of IL-3 autocrine tumors in vivo. Autocrine IL-3 expression in class-1 tumors results from increased IL-3 mRNA stability due to the loss of negative *trans*-acting posttranscriptional control. This defect can be corrected by somatic cell fusion to the nontumorigenic parental PB-3c resulting in downregulation of oncogenic IL-3 expression and concomitant tumor suppression. Expression of the v-H-ras oncogene remains unaffected in class-1 hybrids. In contrast, class-2 tumors are characterized by transcriptional activation of the IL-3 gene due to the insertion of an endogenous retroviral element (intracisternal A-particle) which cannot be overcome by cell fusion (see Hirsch *et al.*, 1993, *J.Exp.Medicine* 178, 403-411). Although v-H-ras is required for generation of either class of tumors, expression of anti-sense ras constructs inhibited only the proliferation of class-1 tumor cells. Experiments are underway to characterize the target and the nature of the class-1 alteration.

N 111 EFFECT OF EGF AND TGF-β1 ON SER/THR PROTEIN PHOSPHATASE ACTIVITY IN CCL64 MINK LUNG EPITHELIAL CELLS, Richard E. Honkanen[1],Tsun S. Ku[1] and Philip H. Howe[2]. [1]Department of Biochemistry, University of South Alabama, Mobile, AL 36688, and [2]Department of Cell Biology, Cleveland Clinic Research Institute, Cleveland, OH 44195.

CCL64 mink lung epithelial cells are a well characterized model system for studying the mechanisms of TGF-β1 mediated growth arrest. Synchronized G0-arrested CCL64 cells undergo cell cycle traverse upon stimulation with 10% fetal bovine serum (FBS) and or 10-20 ng/ml epidermal growth factor (EGF). The addition of TGF-β1 to EGF/FBS stimulated CCL64 cell cultures reversibly arrest growth in late G1 at the G1/S-phase boundary. In other cell lines TGF-β1 has been linked to the maintenance of Rb in an under phosphorylated (tumor suppressing) state, and a considerable amount of effort has been devoted to the identification of the protein kinases that are inhibited by TGF-β1. However, the maintenance of Rb in a under phosphorylated state could also be mediated by the regulation of a serine/threonine protein phosphatase(s).

In this study some recently identified potent and specific inhibitors of serine/threonine protein phosphatases (cantharidin and cypermethrin), some established inhibitors (okadaic acid and microcystin-LR), and a extract with an inhibitory activity which we have yet to definitively identify were utilized to characterize the ser/thr protein phosphatase activity in normal and transformed CCL64. These inhibitors were then used to study the relationship of ser/thr protein phosphatase activity, EGF/FBS stimulated cell growth and TGF-β1 induced growth arrest. Our studies suggest that a serine/threonine protein phosphatase plays an important role in the regulation of cell cycle progression in CCL64 cells.

N 112 CODON CASSETTE MUTAGENESIS: A NEW METHOD OF SITE-DIRECTED MUTAGENESIS, Deena M. Kegler-Ebo, Catherine Docktor, and Daniel DiMaio, Department of Genetics, Yale University School of Medicine, New Haven, CT 06510

We have devised codon cassette mutagenesis, a two-step method of site-directed mutagenesis to insert or replace single codons at a specific site in a DNA molecule. In the first step, a target molecule is constructed that contains a blunt-end at the site of codon insertion. In the second step, a double-stranded mutator oligonucleotide cassette is inserted at the target site and then resolved to yield the codon insertion. Each mutagenic cassette contains a direct three basepair repeat at its ends and two head-to-head recognition sites for the restriction endonuclease Sap I, an enzyme that cleaves outside of its recognition site. The Sap I recognition sites in the mutagenic cassette are oriented so that digestion removes most of the cassette, leaving only a three base overhang that is ligated to generate the final mutation. Each cassette can insert two different codons, depending on the orientation in which it is inserted into the target molecule. Using a derivative of pUC19 plasmid DNA, we have constructed both codon insertion and substitution mutations by using this method. Because the mutagenic cassette is removed during the resolution step, the same cassette can be used at all target sites. Thus mutagenic cassettes are "off the shelf" reagents that can be used to insert or replace codons at any constructed target site. By using 11 different mutagenic cassettes that in aggregate insert all twenty codons, this methodology should be a particularly useful and inexpensive approach for replacing one amino acid with all 19 substitutions without the need for synthesizing nineteen different mutagenic oligonucleotides or for extensive screening following mutagenesis with degenerate oligonucleotides. Our laboratory is investigating the molecular mechanisms underlying the transforming activity of the bovine papilloma virus E5 protein that binds to and activates the PDGF ß receptor in transformed fibroblasts. In an effort to better understand the mechanism of action of the E5 protein, the E5 gene will be the first biological target for mutagenesis via codon cassette mutagenesis.

N 113 THE MURINE INTERFERON REGULATORY FACTOR 1 (IRF-1) IS A NEGATIVE REGULATOR OF CELL PROLIFERATION, Sabine Kirchhoff, Antonis E. Koromilas* and Hansjörg Hauser, *Genetics of Eukaryotes, GBF - Gesellschaft für Biotechnologische Forschung mbH, D-38124 Braunschweig, F.R.G., *Department of Biochemistry, McGill University, Montreal, Quebec H3G 1Y6, Canada*

Besides their ability to render cells resistant to virus replication, Interferons (IFNs) play a role in the regulation of the immune response, as differentiation factors and as cell growth inhibitors. Interferon regulatory factor 1 (IRF-1) is a nuclear DNA-binding factor which recognizes a dimeric hexanucleotide sequence that is present in the ß-interferon promoters (PRDI) as well as in regulatory sequences (ISREs) of most IFN-inducible genes, including MHC class I genes. Its expression is induced by IFN-ß, viruses and a series of regulatory cytokines.

Recently, IRF-1 was recognized to act as a tumor-suppressor. Since its overexpression is not tolerated in mammalian cells we have established two different systems for conditional activation of IRF-1 expression and activation, respectively. In one case, an inducible promotor was used, in the other case fusion proteins composed of IRF-1 and the hormone binding domain of the human estrogen receptor was used. Both systems allow to measure the effect of IRF-1-protein on cell proliferation and on activation of downstream genes. IRF-1 activity results in the induction of a series of Interferon-induced genes, IFN-ß and the activation of the double-stranded RNA-induced kinase (dsI). Recently it was shown that overexpression of a trans-dominant negative mutant of this kinase results in tumor formation in nude mice. Our results suggest that dsI induction is involved in the antiproliferative effect of IRF-1 since the expression of the trans-dominant negative mutant releaves the negative effect of IRF-1 in NIH 3T3 cells.

N 114 ENDOGENOUS MOUSE P53 PROTEIN GENERATED BY ALTERNATIVE SPLICING, Molly Kulesz-Martin, Yuangang Liu and Yu Wu, Grace Cancer Drug Center & Dept. of Experimental Therapeutics, Roswell Park Cancer Institute., Buffalo, NY 14263

We have demonstrated previously that a wild type alternatively spliced p53 (p53as) RNA exists in cultured cells and normal tissues at approximately 30% of the major p53 RNA form [Han and Kulesz-Martin, *Nucleic Acids Res.*, 20:1979-81, 1992]. The protein expected to be encoded by the p53as transcript differs in 17 carboxyl terminal amino acids and is truncated by 9 amino acids due to alternative splicing of intron 10 of the p53 gene. Specific polyclonal antibody to a synthetic peptide unique to this p53as transcript has been generated and used to detect p53as protein in nontransformed and malignant epidermal cells. The p53as immunoreactivity was observed in approximately 1% of the cells, was nuclear and was preferentially expressed during the G2 phase of the cell cycle and in cells with >G2 DNA content. Most p53as(+) cells also were p53(+). In contrast, p53 immunoreactivity was present in 3 to 10% of the cells and was preferentially expressed during G1 as expected. In view of the dimerization and tetramerization of p53 protein thought to be necessary for activity in DNA binding or transcription, certain functional properties of p53 and p53as proteins are being compared. Each protein was produced in rabbit reticulocyte lysates and reacted with specific antibody to the major form of p53 only (PAb421) or p53as only (ApAs), respectively. Both proteins bound to PAb246, implying that p53as can exist in the wild type conformation, consistent with a tumor suppressor function. Equal amounts of lysates containing p53as protein alone or p53as cotranslated with p53 demonstrated distinct bands after binding to a ^{32}P-labelled, p53-specific DNA binding sequence when compared to lysates containing p53 protein alone. Because p53as retains the acidic C-terminal amino acids important for dimerization, the p53as-specific band may represent homodimers of p53as or heterodimers of p53as and p53 proteins. A model is proposed in which p53as and p53 proteins associate with each other to form dimers and oligomers with altered sequence specificity and/or efficiency of DNA binding, with consequences for differential p53 function in cells.

N 115 ROLE OF RAF IN MALIGNANCY: CONTROL OF RAF GENE EXPRESSION BY NEGATIVE GROWTH EFFECTORS. Jong-Eun Lee,[1] Thomas W. Beck,[1] Frank J. Rauscher III[2] and Ulf R. Rapp.[3] [1]BCDP, Program Resources Inc./Dyncorp, NCI-FCRDC, Frederick, MD 21702, [2]The Wistar Institute, Philadelphia, PA 19104 and [3]Laboratory of Viral Carcinogenesis, NCI-FCRDC, Frederick, MD 21702.

Overexpression of wild-type human raf proto-oncogenes in NIH 3T3 cells fails to produce a transformed phenotype, however, if overexpressed with wild-type ras these normal genes cooperate in NIH 3T3 cell transformation. Ras and raf overexpression occurs in a variety of tumors. Thus gene products which coordinately regulate the expression of human proto-oncogenes are likely to be important in the initiation and/or maintenance of cancer cell phenotype and gene products which down-regulate proto-oncogene expression would be candidate tumor suppressors.

To identify factors which may regulate Raf gene expression, we have cloned and sequenced promoter regions for human Raf-1 and A-raf genes and mapped their transcriptional initiation sites. The Raf-1 promoter is located in a CpG island whereas the A-raf promoter in a region having a low G+C content. The initiation sites contain near consensus TFII-I binding sites. Sequence elements located in both the Raf-1 promoter and the A-raf promoter contained sequence similarity to Wilms tumor (WT1) and early growth response elements. The A-raf promoter region also contains multiple hormone response elements (HREs). To determine whether these sequences might be targets for regulation by these gene products, we used purified proteins in EMSAs to detect specific binding and co-transfection assays with wild-type and mutant expression vectors with Raf promoter/reporter constructs. The results indicate these promoters are regulated by factors associated with differentiation.

N 116 THE RETINOBLASTOMA PROTEIN IS REQUIRED FOR CELL CYCLE REGULATORY FUNCTION OF CYCLIN D1, Jiri Lukas, Heiko Muller , Anne A. Kjerulff, Michael Strauss and Jiri Bartek, Division for Cancer Biology, Danish Cancer Society, DK-2100 Copenhagen, Denmark

The retinoblastoma tumour suppressor gene product (pRB) appears to play a growth-restraining role by participating in regulation of G1 progression of the vertebrate cell division cycle. pRB exerts its function, and in turn is regulated, via cell cycle-dependent complex formation with numerous cellular regulatory proteins including the potentially oncogenic cyclin D1. Consistent with the emerging functional interplay between pRB and D-type cyclins, we now report that cyclin D1 protein shares with pRB the ability to be tightly associated with a nuclear structure in the G1 phase of the cell cycle. Our immunoblotting analysis of a wide range of normal and tumour-derived cell types revealed grossly reduced cyclin D1 expression in cancer cell lines containing mutated retinoblastoma (RB) gene, as compared with considerably higher cyclin D1 abundance in either normal cells or tumour cell lines carrying wild-type RB gene and functional pRB. In addition, antibody-mediated cyclin D1 knockout experiments demonstrate that the cyclin D1 protein, normally required for G1 progression, is dispensable for passage through the cell cycle in cell lines which have suffered loss-of-function mutations of the RB gene. The results suggest that pRB serves as a major target of cyclin D1's cell cycle regulatory function. Based on available data including this and the accompanying studies, we propose a model for an 'autoregulatory loop' mechanism, featuring pRB and cyclin D1 as both mutual regulators and targets of regulation, aimed at control of G1 phase progression in cycling mammalian cells.

N 117 REGULATION OF THE SUSPECTED TUMOR SUPPRESSOR GENE PRODUCT CLP IN HUMAN BREAST EPITHELIAL CELLS, Shrikant Mishra, David Zacharias, and Emanuel E. Strehler, Department of Biochemistry and Molecular Biology, Mayo Graduate School of Medicine, Mayo Clinic, Rochester, MN 55905

Calmodulin (CaM)-like protein (CLP), coded by an intronless gene located on human chromosome 10p13-ter, has recently been implicated as a putative tumor suppressor gene product in human breast cancers of epithelial origin. In contrast to the ubiquitous expression of calmodulin transcripts and protein, CLP mRNA and protein expression are restricted to cells of pseudostratified and stratified epithelium. mRNA and protein expression of CLP was found in normal breast epithelial cells and completely lacked in transformed cells. As known tumor suppressors like the retinoblastoma gene product (Rb) have been implicated in regulation of the cell cycle, specifically at the G_1/S boundary, we sought to examine whether CLP expression is in any way linked to or regulates the cell cycle. CLP mRNA levels were analyzed by reverse transcriptase PCR in normal and transformed epithelial cells synchronized by a reversible nocodozole block. The CLP gene was cloned in the sense and antisense orientation into the eukaryotic expression vector PCB6 driven by a CMV promoter (additionally inducible by 1 mM sodium butyrate). We are currently studying the CLP mRNA and protein levels by Northern blotting, reverse-transcriptase PCR, and phenylsepharose affinity extraction in normal and transformed breast epithelial cells synchronized by a reversible nocodozole block. Using cells transfected with the CLP sense and antisense construct, we are beginning to investigate whether CLP, like CaM, can: 1) modify the length of duration of the cell cycle, specifically the G_1 phase and, 2) modulate the activity of cell cycle specific phosphatase. We hypothesize that regulated expression of CLP at the G_1/S boundary could be critical in understanding the molecular basis of action of CLP as a potential tumor suppressor gene product.

N 118 p53 CONTROL OF CELL CYCLE PROGRESSION, Michael Mowat, Nancy Stewart, Geoff Hicks, David Litchfield, and Frixos Paraskevas, Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Manitoba, Canada R3E 0V9.

Wild type p53 is capable of inducing cell cycle arrest at specific points in the cell cycle, an ability lost by most p53 mutants. A recent report established that the first block occurs at the R point before commitment to DNA replication (S) (1). We have identified a second p53 induced arrest point found at G2/M. Cell lines expressing a temperature sensitive mutant, p53val^{135} alone or with EJras were synchronized by serum starvation and then shifted to the wild-type-p53val^{135} conformation conditions (32°C) 18 hours post release into the cell cycle (late G1-S phase). The cell populations were harvested at varying times afterwards and analyzed. DNA flow cytometry analysis confirmed that cells expressing p53 at 32°C arrested in both G1 and G2/M, where as cells left at the permissive temperature continued to progress through the cell cycle. We are currently completing a second analysis on the same cell lines pulsed labelled with bromodeoxyuridine (S phase) before the temperature shift. Preliminary results indicate that a significant proportion of the S phase labelled cells arrest in G2/M as well as G1 at the nonpermissive temperature, but not in S phase. Phosphorylation by casein kinase II has recently been demonstrated to be required for the activation of wild type p53 growth suppressive and binding activities (2,3). Our interest is whether this association changes during specific points of the cell cycle, possibly affecting the function of either component in cell cycle control. To address this, we used cell populations synchronized by serum starvation(G0) or by the drugs hydroxyurea(G1/S) and nocadozole(G2/M). Cell populations were analyzed for p53/CKII complex formation and kinase activity. Our results indicate that p53/casein kinase II complexes and associated kinase activity are found preferentially, but not exclusively, in G2/M. Populations of cells arrested by wild type p53 expression using the temperature sensitive p53val^{135}, have also been studied for p53/casein kinase II complex formation. Our results indicate that there is complex formation and associated kinase activity with both mutant and wild type p53 in both G1/S and G2/M populations. The G2/M arrested population possess greater p53 associated casein kinase II activity (>2 fold difference).

1. Lin, David et al. (1992) PNAS 89, 9210-9214.
2. Milne, D.M. et al. (1992) Nucleic Acid Research 20, 5565-5570.
3. Huppe, T.R. et al. (1992) Cell 71, 875-886.

N 119 **Isolation and characterization of a novel TGF β1-inducible and *ras*--recision gene (HIC-5) that encodes putative Zn-finger motifs.** Kiyoshi Nose, Motoko Shibanuma, Jun'ichi Mashimo and Toshio Kuroki*. (Department of Microbiology, Showa University School of Pharmaceutical Sciences, Hatanodai 1-5-8, Shinagawa-ku, Tokyo-142, Japan; *Department of Cancer Cell Research, Institute of Medical Sciences, University of Tokyo, Shirokanedai, Minato-ku, Tokyo-108, Japan)

Transforming growth factor (TGF) β1 is a potent inhibitor of growth in mouse osteoblastic MC3T3-E1 cells. To isolate genes that are involved in growth-inhibition by TGF β1, the differential screening method was adopted using cDNA libraries constructed from cells treated with TGF β1 for 4 hr. Six independent cDNA clones were isolated whose expression was induced by TGF β1 with peaks of 6-10 hr. From sequence analysis, one of them (HIC-5) seemed to encode a novel protein that had seven Zn-finger motifs with a molecular weight of 50 kDa. Expression of HIC-5 was found to be decreased significantly in*ras*-transformed MC3T3 cells. Its expression was almost completely extinguished also in NIH 3T3 cells transformed by v-ki-*ras* (DT cell line), and was recovered to normal levels in its flat revertant (C11). Antibody raised against synthetic peptide of HIC-5 protein immunoprecipitated a nuclear protein with an apparent molecular weight of 50 kDa. Among mouse organs, expression of HIC-5 was detected mostly in lung and macrophage. Nucleotide sequences of HIC-5 were thought to be conserved between mouse and human, since mouse cDNA cross-hybridized with human genomic DNA and mRNA. Basal levels of HIC-5 mRNA were significantly decreased in several human tumor-derived cell lines. On the other hand, its expression was enhanced in normal human fibroblasts in senescence compared to that in young cells. Mammalian expression vectors of HIC-5 were constructed using the CMV-promoter, and transfected into MC3T3 cells together with neo-resistant gene. Colony formation of neo^r cells was decreased with increasing amounts of HIC-5 expression plasmid. These results indicate that HIC-5 has some roles in negative growth regulation, and a possible candidate of a new member of tumor suppressor genes. Ref: Shibanuma et al., *Eur. J. Biochem.* (in press).

N 120 ACTION OF TUMOR SUPPRESSORS THAT RESTRICT CUMULATIVE PROLIFERATIVE CAPACITY, **Russell D. Owen** and J. Carl Barrett, Laboratory of Molecular Carcinogenesis, NIEHS, NIH, Research Triangle Park, NC 27709

Primary cultures of normal diploid fibroblasts have a finite lifespan in culture; this characteristic is termed cellular senescence. Several lines of evidence demonstrate that senescence is a genetically programmed process that acts in a dominant fashion to arrest the cell cycle before S phase. Loss or relaxation of senescence is required for characteristic tumor growth; therefore, senescence genes are tumor suppressors. Our laboratory has found several molecular changes associated with the onset or maintenance of cellular senescence, including alterations in the phosphorylation state of RB, a cdc2 substrate. We have found that cdc2 message levels are reduced in senescent cells and that cdc2 protein phosphorylation is controlled in a dominant fashion.

We are investigating the regulation of both kinases and phosphatases with respect to control of cell proliferation. Treatment of non-proliferative cells with okadaic acid, an inhibitor of serine-threonine protein phosphatases, results in a partial relief of cell cycle arrest. Using an in vitro assay of cell-free extracts, we have found that senescent cells contain more protein phosphatase 2A (PP2A) activity per cell than either proliferative or quiescent young cells. This increase does not correspond to a change in PP2A message levels. We are using western blot analysis to determine if the change in activity corresponds to an increase in the amount of catalytic subunit. We are also using an antisense cDNA approach to assay the effects of perturbing the expression of PP2A catalytic subunit upon regulation of proliferation.

N 121 POSITIVE AND NEGATIVE REGULATION OF EPITHELIAL CELL PROLIFERATION BY EPIDERMAL GROWTH FACTOR (EGF) AND TRANSFORMING GROWTH FACTOR-ß1 (TGFß1), Daniel J. Satterwhite, and Harold L. Moses, Vanderbilt University School of Medicine, Nashville, TN 37232-2175

The concept of positive and negative regulation of normal cellular growth by diffusible factors is well illustrated by the effects of EGF and TGFß1 on mouse keratinocytes (MK). MK cells are a non-transformed continuous cell line that becomes quiescent in culture if maintained in EGF deficient media with low serum (0.1%). When restimulated with EGF (4ng/ml) and serum (8%), quiescent MK cells reenter the cell cycle in early G1 in a synchronized fashion. TGFß1 (10ng/ml) will reversibly inhibit the wave of DNA synthesis occurring 12 hours following restimulation of these quiescent cells when added at any point prior to the G1/S boundary. Many carcinoma cells have lost this growth inhibitory response to TGFß1. Recent studies have identified a large number of growth factor inducible genes that are critical regulators of growth in G1 and at the G1/S transition, and are often found to be disregulated in cancer. These include cyclins and their associated kinases as well as transcription factors that positively affect growth, and tumor suppressor genes that negatively affect growth. Previously, work in our lab has shown that suppression of RNA expression of the proto-oncogene c-myc is important in the TGFß1 growth inhibition pathway. Using Northern analysis of EGF restimulated MK cells we characterize the cell cycle specific induction of numerous growth regulatory genes and identify B-myb and cyclin A as potential target genes in the TGFß1 growth inhibition pathway.

N 122 MECHANISMS OF NEGATIVE GROWTH CONTROL: LYN SIGNALS CELL CYCLE ARREST BUT NOT APOPTOSIS. Richard H. Scheuermann*, Emilian Racila§, Thomas Tucker§, Louis J. Picker* and Jonathan W. Uhr§. Laboratory of Molecular Pathology and Department of Pathology*, and Department of Microbiology§, University of Texas Southwestern Medical Center, Dallas, TX 75235-9072.

Protein products of most tumor suppressor genes probably function in some capacity as negative regulators of cell growth. We have been interested in the relationship between regulators of cell growth and the phenomenon of tumor dormancy, where cancer cells are maintained for long periods of time in a quiescent state. Clinical examples of tumor dormancy have been well established for many human malignancies. We have chosen the mouse B cell lymphoma BCL_1 as a model system to study tumor dormancy. BCL_1 expresses membrane-bound IgM and grow rapidly in the spleens of recipient mice. However, the tumor can be induced into a dormant state *in vivo* either by prior immunization with BCL_1 Ig in syngeneic mice, or by the addition of anti-Ig antibodies to SCID recipients. Dormant lymphoma cells differ from growing lymphoma cells in morphology, cell cycle parameters, and oncogene expression profiles. These observations suggest that an important aspect of dormancy induction is the initiation of a signal transduction cascade by cross-linking of membrane Ig (mIg). In addition to immunoglobulin heavy and light chains, the mIgM complex contains the products of the mb-1 (IgMα) and B29 (Igß and Igγ) genes. These associated molecules apparently function to connect the predominantly extracellular Ig to the inside of the cell through a group of src-family tyrosine kinases (TKs) - lyn, fyn, blk and lck, which have been found associated with mIg by co-immunoprecipitation. Activation of one or more of these TKs is likely to be responsible for the signal transmission.

We have found that anti-Ig induces an anti-proliferative response in BCL_1 cells, which includes the induction of both cell cycle arrest and apoptosis. Treatment of BCL_1 cells with specific antisense oligonucleotides results in the depletion of the lyn tyrosine kinase protein. When depleted cells are challenged with anti-Ig, apoptosis is induced as in controls, however anti-Ig is no longer able to induce cell cycle arrest. This indicates that the lyn tyrosine kinase is responsible for transduction of the cell cycle arrest signal initiated by mIg cross-linking, and define a branchpoint in the cytosolic signaling pathways mediating cell cycle arrest and apoptosis. Since lyn functions to signal cell cycle arrest, it may serve a role in normal growth regulation as a tyrosine kinase tumor suppressor protein. Experiments to test this hypothesis will be presented.

N 123 DIFFERENTIAL ANALYSIS OF THE EFFECTS OF TRANSFORMING GROWTH FACTOR β ON CELL GROWTH IN VITRO IN RB-RECONSTITUTED AND PARENTAL BLADDER CARCINOMA CELLS

Rei Takahashi[1], Motowo Nakajia[2], Liang Ping[1], Mitsuhiko Osaka[1], Hironori Haga[1], William Benedict[3], Um Soon Ho[4], Taketoshi Sugiyama[1]. Depts. of Tumor Biology (Pathology)[1] and Geriatric Med[4]. Kyoto Univ., JAPAN, [2]The Institute of Applied Microbiology, Tokyo Univ., JAPAN, [3]Center for Biotech. Baylor College of Med. The Woodlands, TX.

The function of the human retinoblastoma (RB) gene was found to be linked to cell growth in vitro and in vivo. To further analyze the pathways in which the RB gene is involved, we established an RB-reconstituted cell line by transfecting an RB-expression plasmid by the calcium phosphate method. Human bladder carcinoma (HTB9) cells stably reconstituted with the wild type RB gene were analyzed *in vitro* under TGFβ-stimulated conditions. Not only the growth rate in mass culture but also the plating efficiency and colony formation in soft agar of RB+ HTB9 cells were more severely suppressed after the treatment of TGFβ. TGFβ-induced expression of matrix metalloproteinases (MMPs) was markedly enhanced in RB-reconstituted cells compared to the parental cells. The effects of TGFβ on RB+ HTB9 cells were reduced in the presence of EGF in a dose dependent manner. In order to isolate genes involved in the pathways, differential display of cDNAs from TGFβ-responsive and non-responsive cells is to be analyzed.

N 124 Regulation of the Retinoblastoma protein by MAP kinase

Dennis J. Templeton, Minhong Yan, Louis A. Parrott, Lynn E. Horton and Yongyi Qian, Institute of Pathology, Case Western Reserve University, 10900 Euclid Avenue, Cleveland Ohio 44106

The Retinoblastoma tumor suppressor protein (pRb) is a nuclear growth control protein that is regulated by phosphorylation that varies through the cell division cycle. Phosphorylation of pRb also increases after mitogenic stimulation of cells. Since the cytoplasmic kinase termed MAP kinase (Mitogen Activated Protein kinase, MAPK) is stimulated after many mitogenic signals, we tested whether hyperphosphorylation of pRb can result from MAPK activation. We have found that activated MAPK(p42) increases pRb phosphorylation *in vivo*, and that MAPK can phosphorylate pRb *in vitro* on sites similar, but not identical, to those phosphorylated by a cyclin directed kinase. Activation of the MAPK pathway negates the ability of pRb to inhibit growth of Saos-2 cells, and eliminates the ability of pRb to interact with the E1A oncoprotein. Thus, regulation of pRb phosphorylation and function can result from a direct interaction with MAPK, and may reflect control mechanisms distinct from regulation by cyclin directed kinases.

N 125 MODULATION OF THE PROPERTIES OF p53 BY MDM2, AND RAS BY ETS. Bohdan Wasylyk, Moussa Alkhalaf, Ladislav Andera, Marie-Christine Dubs, Antoine Giovane, Soonjung L. Hahn, Thierry Leveillard, Sauveur-michel Maira, Alexander Pintzas, Peter Sobieszczuk and Christine Wasylyk. LGME-CNRS, U184-INSERM, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg cedex, France.

The activity of the tumor suppressor p53 is modulated by interactions with the mdm2 oncoprotein. We will describe our studies on the mechanisms of this regulation and the interaction of mdm2 with other factors. Transformation by the Ras oncogene is mediated by nuclear factors. We have shown that: 1). The Ets transcription factor is an important component of the Ras signalling pathway, since trans-dominant mutants of Ets revert Ras transformed cells. 2). A new member of the Ets family, NET, is highly expressed in transformed cells. It is a negative regulator of transcription that is switched to an active form by Ras expression. 3). The oncoprotein vEts has an altered C-terminus that affects its trans-activating properties in various ways and its sensitivity to signalling pathways induced by oxidative stress.

N 126 FUNCTIONAL ANALYSIS OF THE TGF-BETA TYPE II RECEPTOR

Rotraud Wieser, Liliana Attisano, Jeffrey L. Wrana, and Joan Massagué Memorial Sloan Kettering Cancer Center and Howard Hughes Medical Institute, 1275 York Ave, New York, New York 10021.

TGF-β is a potent inhibitor of cell growth in many cell types, and loss of responsiveness to this cytokine is associated with malignant transformation in several cases. The three most widely distributed receptors for TGF-β, receptor types I, II, and III, have been cloned recently. Both the type I and type II receptors are transmembrane kinases with homology to the serine threonine kinase subfamily. They form a heteromeric complex which transduces the TGF-β signal to the cell.

In order to gain insight into the mechanism of signal transduction, we introduced various mutations into the TGF-β type II receptor (TβR-II). We either replaced or deleted the following regions: the juxtamembrane region, inserts 1 and 2 in the kinase domain, and the serine threonine rich C-terminal tail. Corresponding regions in tyrosine kinase receptors have been shown to contain sequences crucial for interaction with substrates. The resulting molecules were transfected into mutant derivatives of Mv1Lu cells that have lost responsiveness to TGF-β due to mutations in TβR-II. TGF-β responses can be restored to these cells by transfection of the cloned type II receptor. Analysis of the TβR-II mutants indicates that none of the intracellular regions mentioned above is necessary for the interaction of TβR-II with TβR-I. In addition, the juxtamembrane region, insert 1 and the C-terminal tail appear not to be required for signaling the TGF-β responses tested. In contrast, insert 2 is required for all these responses to occur. However, this appears to be due to the fact that it is necessary to support kinase activity of the receptor rather than it being an interaction site for substrates Therefore, substrate recognition sites of the TGF-β receptor complex must be located in the TβR-II kinase region itself or in TβR-I. These possibilities are currently under investigation.

N 127 *DROSOPHILA* TUMOR SUPPRESSOR GENES AND THEIR HUMAN HOMOLOGS. Daniel F. Woods, Robin W. Justice, Kellie L. Watson, and Peter J. Bryant, Developmental Biology Center, University of California, Irvine, CA 92717

In *Drosophila*, a large number of loci now have been identified in which loss-of-function mutations lead to overproliferation and are therefore called tumor suppressor genes (tsgs). Several of these tsgs have been cloned and found to encode proteins that also have homologs in humans. Here we present data on four of these *Drosophila* tsgs. Mutations in the *discs large* gene lead to neoplastic overgrowth of imaginal discs, causing the cells to lose apical/basal polarity and preventing them from differentiating. The Discs large protein contains three distinct peptide domains and is the first identified member of a new family of signalling proteins. Additional members have been cloned in a number of different systems including humans. Recessive lethal mutations at the *fat* locus cause hyperplastic imaginal disc overgrowth, in which the imaginal disc cells retain their epithelial structure, junctional complexes and ability to differentiate. However, defective adhesion is suggested by the separation of epithelial vesicles from the epithelial sheet. The gene encodes a giant relative of cadherin cell adhesion molecules, but it has a novel cytoplasmic domain. A new tumor suppressor gene, named *warts*, has been identified by screening for overgrowth in mitotic recombination clones. Loss of *warts* function leads to clone rounding, splitting, and overgrowth. In epithelial tissue lacking *warts*, cuticle is deposited between cells as well as over their apical surfaces, suggesting an abnormality in cell-cell contact. The Warts protein is apparently a *Drosophila* homolog of a recently cloned human protein kinase. Finally, we have found that mutations in the RpS6 locus of *Drosophila* cause blood cell neoplasia. These results show that the *Drosophila* model system can be used effectively to identify and characterize novel tsgs that have human homologs.

N 128 RETROVIRAL INTERRUPTION OF CHROMOSOMAL GENES PREFERENTIALLY ACTIVE DURING SERUM STARVATION IS ASSOCIATED WITH GROWTH ARREST AT LOW CELL DENSITY, Chih-Jian Lih[1*], Yu-Lun Huang[1], Hsin-Fang Yang-Yen[1], Annie. C.Y. Chang[2], Stanley N. Cohen[1,2] and Sue Lin-Chao[1*], [1]Institute of Molecular Biology, Academia Sinica, Nankang, Taipei, Taiwan, [2]Departments of Genetics and Medicine, Stanford University School of Medicine, Stanford, CA

Use of a self-inactivating retrovirus carrying lacZ-based reporter gene to identify mammalian genes induced by cell growth-arrest has been described previously by Brenner et al. (PNAS, 86: 5517-5521, 1989). We found that independent retroviral insertions into a single chromosomal allele of each of four growth-arrest-specific (gas) genes are associated with the cessation of cell division at low cell density in cultures maintained in 10% serum. Arrested cell growth occurred at 20% to 30% the density required to stop growth of the parental NIH3T3 cell line; furthermore, greater than 60% of the population of retrovirally mutated cells seeded at a density of 1×10^4 cells per cm^2 were arrested in G1/G0 48 hours after seeding and the fraction of cells in G1/G0 at 48 hours increased to 90% when cells were seeded at a density of 4 to 5×10^4 cells per cm^2. In contrast, even at the highest seeding cell density tested (6.2×10^4 cells per cm^2), less than 60% of cells from the parental cell line had arrested in G1/G0 during the same time interval, reflecting the ability of the parental line to multiply at cell densities that prevent growth of the insertionally mutated lines. Additionally, cell lines carrying retroviral insertion in gas genes showed dramatically reduced transformation by v-ras oncogene, suggesting that the genes interrupted by the insertion may act downstream of v-ras in the oncogenic pathway. We suggest that insertional mutation of a single allele of these gas genes may lead to growth arrest at reduced cell density by decreasing the intracellular concentration of gas gene products required for continued cycling. The generality of the effect on oncogenic transformation is under investigation.

Cell Cycle Replication Transcription and Development

N 200 THE TRANSCRIPTION FACTOR E2F MAY PLAY A ROLE IN CELLULAR SENESCENCE, Cynthia A. Afshari and Maria Mudryj, Center for the Study of Aging and Human Development, Division of Geriatrics, Department of Medicine, Duke University Medical School, and GRECC, VAMC, Durham, NC 27705

Normal, diploid fibroblasts in culture have a finite lifespan after which they undergo a process termed cellular senescence. It is possible that genes that serve a growth suppressive/tumor suppressor gene function may normally be involved in this process. Senescence most likely involves the regulation of two classes of genes, the suppression of proliferation-promoting genes as well as the activation of anti-proliferative genes. The expression of these genes could be under the control of various transcription factors. Our study focuses on the transcription factor E2F that has been shown to complex with several cellular proteins including the product of the RB tumor suppressor gene, p107, cyclin A, and cdk2. E2F binding sites are present in the promoters of several genes that are required for DNA synthesis such as thymidine kinase, dihydrofolate reductase, cdc2, and thymidylate synthetase. Therefore, changes in the regulation of E2F activity may be important in cellular senescence and cessation of DNA synthesis. We have examined the E2F complexes that are present in serum stimulated young versus aged human fibroblasts and have found that E2F complexes do exist in senescent cells and are similar but not identical to complexes found in young, G_0-arrested fibroblasts. We are currently examining these complexes.

N 201 THE EFFECT OF EXPRESSION OF WT1 IN TRANSFORMED FOETAL KIDNEY CELLS, Gale Brightwell, Karim T.A. Malik, Veronique Poirier and Keith Brown, Department of Pathology and Microbiology, School of Medical sciences, University of Bristol, U.K.

WT1 is a tumour suppressor gene which is mutated in Wilm's tumours. The gene encodes a zinc-finger DNA-binding protein and is specifically expressed in foetal kidney cells and a few other tissues. This would imply that the protein is involved in kidney differentiation.

A full length WT1 cDNA was cloned from a human foetal kidney cDNA library. This clone constituted a +51bp, -KTS splice variant encompassing the entire coding region. The WT1 cDNA was subcloned into the expression vector pCMV and subsequently transfected into 293 cells, an adenovirus transformed human embryonic kidney cell line. The presence of the transfected gene and transcript was verified by Southern and Northern blotting respectively. The effects of expression of both sense and anti sense orientations in 293 cells are currently under investigation. Results of growth rate assays and changes in immunological marker status are presented.

Initial results would suggest that there was no marked effect either on the expression of p53, N-CAM, IGF2 and cytokeratin or on the cellular growth rate of transfected cell lines. 293 cells containing either sense or anti sense WT1, like untransfected cells, induced tumour formation in nude mice, one of which showed an altered pattern of differentiation.

N 202 IDENTIFICATION OF A HUMAN HOMOLOG OF NUC2 THAT INTERACTS WITH THE RETINOBLASTOMA PROTEIN, Phang-Lang Chen, Yi-Chinn Ueng and Wen-Hwa Lee, Center for Molecular Medicine/Institute of Biotechnology, University of Texas Health Science Center at San Antonio, Texas, 78245

The RB protein is associates with many cellular proteins to form complexes that may allow it to execute growth regulatory functions. Using the yeast two-hybrid system, about two dozen clones encoding RB-associated proteins have been isolated. Here, we characterize one of them, a full length cDNA clone which encodes an 824 amino acid protein containing ten 34-amino acid repeats characteristic of the TRP protein family. Homology aligment with these repeat sequences showed about 60% sequence identity with the nuc2 protein of fission yeast and bimA protein of Aspergillus. The organization of the ten amino acid repeats in the new protein is also similar to the organization in the two lower eukaryotic genes, suggesting that the newly cloned potein could be the human homolog to nuc2. We have therefore named it H-nuc. This protein binds to unphosphorylated RB in a region similar to the region to which SV40 large T antigen binds. However, the binding region of the H-nuc is mapped to its TPR region, which contains sequences different from either T-antigen or E2F-1. An antibody specific to H-nuc was prepared and recognizes a 90 kd protein with DNA-binding activity. It is also interesting to note that H-nuc maps to chromosome 17q21-22, the region at which a human familial breast cancer gene resides.

N 203 **Inhibition of p53 Gene Transcription in Hela Cells by O-Phospho-L-Tyrosine**

CHEN Zhen Ping and Desmond Chak-Yew YEUNG
Department of Biochemistry, University of Hong Kong

The possible roles of p53 in the regulation of growth and differentiation as well as apoptosis have been studied extensively. However, the literature on the regulatory mechanisms governing p53 gene expression is scanty. Since protein tyrosine phosphorylation is implicated in growth control processes, and as insulin is a well established growth promoting agent in a variety of cell types, we have studied the effects of O-phospho-L-tyrosine (P-Tyr) and insulin on p53 gene expression in Hela cells. Our results showed that while 2 mM P-Tyr alone did not alter p53 gene expression in Hela cells maintained in the presence of serum, the same concentration of P-Tyr in the presence of 10^{-6} M insulin significantly decreased p53 gene transcription, although insulin by itself did not appreciably affect the level of p53 transcription. Whereas in starved cells, neither 2 mM P-Tyr alone nor in the presence of 10^{-6} M insulin altered the level of p53 transcript. However, at a higher concentration (20 mM), P-Tyr alone was able to suppresses p53 gene transcription significantly when the cells were maintained with or without serum. Furthermore, the suppressive effect of P-Tyr was again potentiated by 10^{-6} M insulin.

The observed effects of P-Tyr and insulin may be explained on the basis of their known molecular mechanism of action. As P-Tyr is a universal substrate of protein tyrosine phosphatases, it is logical to expect that its presence at high concentrations leads to a decrease in protein tyrosine dephosphorylation. Acting through its receptor tyrosine kinase, insulin is able to enhance protein tyrosine phosphorylation. Our results thus suggest that protein tyrosine phosphorylation may be involved in the suppression of p53 gene transcription.

N 204 THE REGULATION OF TRANSCRIPTION AND DIFFERENTIATION BY RB, P107 AND CYCLINS, Lisa Crossley[1], Zhaohui Shao[2], Jalila Adnane[2], and Paul D. Robbins[2], Departments of Anesthesiology[1] and Molecular Genetics and Biochemistry[2], University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

The Rb tumor suppressor gene product is able to regulate transcription through direct interaction with specific transcription factors. p107, a protein related to Rb, also appears able to regulate transcription. We have contrasted the ability of Rb and p107 to regulate transcription mediated by specific transcription factors *in vivo* in several different cell types. In contrast to Rb, we have observed no effect of p107 on transcription mediated by Sp1 and ATF-2. Similar to Rb, p107 was able to negatively regulate transcription mediated by E2F. p107 also was able to repress transcription mediated by c-Myc whereas Rb was able to stimulate c-Myc-mediated transcription. To determine if the observed regulation of specific transcription factors by Rb and/or p107 is regulated by cyclins, we have also examined the effects of expression of cyclins in the cotransfection assay. We have observed significant, differential effects of specific cyclins on Rb and/or p107 regulation of E2F, ATF, and Sp1-mediated transcription. These effects of cyclins on Rb and p107-regulated transcription will be presented. We also have been interested in determining the role of Rb and p107 in regulating differentiation. We have developed an assay to examine the effects of expression of specific gene products on differentiation of PC12 cells. PC12 cells are mouse phenochromacytoma cells which able to differentiate into neurons in response to nerve growth factor (NGF). We have observed that both Rb and p107 are able to enhance the response of PC12 cells to NGF. A detailed analysis of the effects of Rb and p107 as well as E2F-1 and cyclins on PC12 differentiation will be presented.

N 205 INTERACTION BETWEEN THE RETINOBLASTOMA PROTEIN AND THE HUMAN HOMOLOGUE OF BRAHMA, A REGULATOR OF HOMEOTIC GENES, Joshua Dunaief, Paul Khavari[1], Sushovan Guha, Kimona Alin, Jeremy Luban, Gerald Crabtree[1], and Stephen Goff, HHMI and Department of Microbiology, Columbia College of Physicians and Surgeons NY,NY 10032 and Unit in Molecular and Genetic Medicine, Stanford University School of Medicine[1], Stanford, CA 94305

Mutations in the retinoblastoma (Rb) gene lead to formation of an invasive retinal tumor and neoplasia in other organs. RB protein binds to c-myc, E2F, and cyclin D, suggesting that RB may influence the cell cycle through these proteins. We have used the yeast two-hybrid system to identify new mammalian partners that bind to RB. We have recovered part of a murine gene, designated Mbrm, that has sequence similarity to Drosophila brahma, an activator of homeotic gene expression, and to the yeast transcriptional activator SNF2/SWI2. The yeast activator is probably part of a multi-protein complex and has homology to DNA helicases. Bacterially expressed Mbrm binds to RB in-vitro, and an Mbrm region required for binding shows similarity to the RB-binding region of the human papilloma virus E7 gene product. Mbrm binds only a small amount of RB from mammalian cell lines containing E7 and other viral oncoproteins, suggesting that the E7 may compete for RB-binding. Conversely, bacterially expressed wild-type RB, but not RB harboring a "pocket" mutation, binds Mbrm and Hbrm, the human homologue. In addition Hbrm and RB co-immunoprecipitate, indicating that Hbrm may mediate effects of RB in vivo.

N 206 THE WT1 GENE PRODUCT FORMS A COMPLEX WITH p53 IN WILMS TUMORS AND IN STABLY TRANSFECTED BABY RAT KIDNEY CELLS

Christoph Englert, Shyamala Maheswaran, Patrick Bennett, and Daniel Haber

Laboratory of Molecular Genetics, Massachusetts General Hospital Cancer Center, Charlestown, MA 02129

The chromosome 11p13 Wilms tumor suppressor gene, WT1, encodes a zinc finger transcription factor whose expression is strictly developmentally regulated in the kidney. Germline mutations in WT1 have been shown to confer genetic predisposition to Wilms tumor, and approximately 10% of sporadic Wilms tumors contain mutations inactivating WT1. To study the biochemical properties of WT1, we developed stably transfected baby rat kidney (BRK) cell lines, expressing high levels of wild-type or mutated WT1 protein. Gel filtration experiments and 2-dimensional electrophoresis demonstrated that the 50kD WT1 protein migrates between 100-150kD under non-denaturing conditions. Immunoprecipitation experiments revealed the coprecipitation of WT1 and p53 proteins from BRK cellular lysates. The physical association of WT1 and p53 proteins could also be demonstrated in protein extracts from sporadic Wilms tumors, using immunoprecipitation followed by Western analysis. The functional significance of the WT1/p53 interaction is suggested by analysis of their transactivational properties. Transcriptional activation by p53 of its target sequence is enhanced four-fold by cotransfection of wild-type WT1, suggesting a cooperative interaction. In contrast, WT1, which represses transcription from its target sequence in the presence of p53, acts as a potent transcriptional activator in the absence of p53. The physical interaction between p53 and WT1 may thus result in the conversion of WT1 from a transactivator to a transcriptional repressor, modulating its effect on potential target genes.

N 207 REGULATION OF p53 BY THE CELLULAR REDOX FACTOR REF-1 *IN VITRO*. Barbara Faha and Nikolai Kley, Department of Molecular Genetics and Cell Biology, Oncology Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ 08543

p53 is one of the most commonly mutated genes in human cancers. Recent data has indicated that p53 functions as a transcriptional regulator, positively regulating genes containing a p53 DNA consensus binding site and negatively regulating certain other genes. Point mutations have been found scattered throughout conserved regions of the p53 protein, most of which induce p53 to adopt an altered immunological phenotype, or "mutant" conformation. Many of these mutant proteins are severely defective in their ability to bind specifically its defined consensus site and to regulate transcription. Under certain circumstances, wild-type p53 may also adopt a "mutant-like" conformation, a shift in phenotype that has been reported to correlate with serum-stimulation of growth arrested cells. What controls such processes is unknown, but changes in phosphorylation of p53 has been suggested to correlate with such changes.

We were interested in studying other post-translational mechanisms that might control p53 function and show here that p53 binding to its consensus DNA binding site *in vitro* is dependent upon its oxidation-reduction state. Oxidation or chemical modification of p53 by sulfhydryl modifying agents such as diamide and N-ethylmaleimide (NEM) eliminates DNA binding and is associated with a shift to a "mutant-like" phenotype as assayed by immunoprecipitation with conformation specific antibodies. Reversible loss of DNA binding and conversion to a "mutant-like" phenotype is also induced by the metal chelator o-phenanthroline (OP), suggesting an important structural role for metal binding cysteinyl residues of p53. Binding to DNA protects p53 from subsequent inactivation by these agents indicating close association of these residues with DNA.

Furthermore, we show that the cellular redox factor, Ref-1, which was demonstrated to stimulate the DNA binding function of AP-1 and other transcription factors, stimulates p53 DNA binding *in vitro*. Purified Ref-1 is also able to reverse the effects of diamide and OP on p53. In addition, Ref-1 is able to restore the DNA binding function of a mutant p53 defective of DNA binding. These results clearly demonstrate that p53 is regulated by oxidation-reduction mechanisms and suggest a novel post-translational mechanism of regulation of activities associated with p53 tumor suppressor function. We are currently characterizing further the action of Ref-1 on p53 and investigating *in vivo* mechanisms of redox regulation of p53 function.

N 208 Structural homology between the Rb protein and the SV40 large T antigen

Yuen Kai T. Fung , Anne T'Ang, Xiang-He Shi, Takashi Koyama, Fu Hui Zhang, Ling Li, GuoLiang Li, Yi Zhao

Departments of Pediatrics and Microbiology, Childrens Hospital of Los Angeles, University of Southern California , School of Medicine , 4650 Sunset Blvd., Los Angeles, California 90027

The retinoblastoma gene, Rb, is a prototype growth suppressor gene, the inactivation of which is associated with tumor development. In our attempt to identify proteins that are associated with the Rb protein, we made the surprising discovery that an anti-Rb antibody Rb1-Ab-16-2 , which was raised against a Rb peptide, P16, can independently immunoprecipitate the SV40 large T antigen . In addition to this antigenically homologous domain , H1, detailed comparison of the sequence of the Rb protein and the SV40 large T antigen revealed the amino acid sequence homology at several other domains. Antibodies raised against a peptide , P9, corresponding to another such domain (H2) of the Rb protein also independently recognize the SV40 large T antigen. That these domains are functionally important is shown by the fact that mutants of the Rb protein at these two domains failed to suppress the growth of cells . Although the function at the molecular level is unknown, we surmise that these structural domains conserved between Rb and the SV40 large T antigen may also be present in cellular proteins that are structurally and /or functionally related to either the Rb protein and /or the SV40 large T antigen. Indeed, immunoprecipitation studies show that the anti-RB antibody Rb1-Ab-16-2 recognizes at least eight cellular proteins of different molecular weights. Candidate cDNA clones corresponding to these cellular proteins were isolated from lambda gt11 libraries using the anti-RB antibody Rb1-Ab-16-2. Initial sequence analysis of three of the clones revealed the existence of structural motifs that are conserved in SV40 large T, Polyoma large T, Rb and the Rb related protein, P107. Since the common thread between these clones is the H1 domain, we propose a model in which the Rb protein (and related proteins) and the CHLAs (cellular homologues of the large T antigen) regulate cell growth by competing for binding to a common target protein(s) through their conserved homology domains.

N 209 TUMOR SUPPRESSOR FUNCTION OF PKR,

Julien Galabru*, Eliane Meurs*, Glen N. Barber§, Michael G. Katze§ & Ara G. Hovanessian*.

*Unit of virology and cellular Immunology, CNRS URA 1157, Institut Pasteur, 75015 Paris, France. §Department of Microbiology, School of Medicine, University of Washington, Seattle, WA 98185.

The RNA-dependent protein kinase (PKR) is a Mr 68,000 protein in human cells (p68 kinase) or a Mr 65,000 protein in murine cells (p65 kinase). PKR is a serine/threonine kinase induced by interferon treatment and generally activated by double-stranded RNAs. Once activated, the known function of this kinase is inhibition of protein synthesis through phosphorylation of the eukaryotic initiation factor 2 (eIF2). Here we have investigated the potential for tumorigenicity in mice of murine NIH-3T3 clones expressing human p68 kinase, either the wild-type or a mutant inactive kinase with a single amino-acid substitution in the invariant lysine-296 in the catalytic domain II. Expression of the mutant p68 kinase was correlated with a malignant transformation phenotype, giving rise to the production of large tumors of at least 1 cm in diameter by 7-12 days in all inoculated mice. In contrast, no tumor growth was observed for several weeks in mice inoculated with NIH-3T3 cell clones expressing either the wild-type recombinant p68 kinase or only the endogenous p65 kinase (the murine kinase). These results suggest that functional p65/p68 kinase (i.e. PKR), by a still undefined mechanism may also act as a tumor suppressor. Consequently, one of the pathways by which interferon inhibits tumor growth might be through its capacity to induce the enhanced expression of this kinase.

N 210 RANDOM OLIGONUCLEOTIDE BINDING SITE OPTIMIZATION AND NON-NUCLEOSOMAL TARGET ISOLATION FOR THE TRANSCRIPTION FACTOR GATA-1, Todd A. Gray and Francis. S. Collins*, Program in Cellular and Molecular Biology, The University of Michigan School of Medicine, Ann Arbor, MI 48109-0650, *Director National Center for Human Genome Research, NIH, Rockville Pike, Bethesda, MD 20892

Sequence specific DNA binding proteins are primarily responsible for regulating the initiation of transcription, the seminal, often rate limiting, step in gene expression. Alterations in gene expression ultimately underlie changes in cellular phenotype and growth characteristics. Increased interest in this area and advances in cloning have given rise to a burgeoning list of cloned transcription factors. Identifying the genomic targets of these factors, such as known tumor supressors and promoters, may shed light on the mechanism of oncogenesis. GATA-1 is a factor which is expressed in erythroid and mast cells and megakaryocytes as well as testis and has been implicated as a key transcription factor for many genes expressed in these tissues. It is essential for appropriate expression of the erythroid expressed globin genes and binding sites for it have been identified in their promoters. As its name implies, the described sites contain a core GATA motif. Through an independent method of identifying functionally important cis sequences that have been conserved through evolution (phylogenetic footprinting (Gumucio et al, MCB,12:4919-29)), binding sites which do not contain this core sequence appear to bind this factor. With this observation supplying the impetus, two unbiased approaches were used to identify high affinity sites as well as potential genomic target sequences. Both rely on selection by immobilized GATA-1, in this case, by a glutathione/GST linkage. Results from random oligonucleotide binding site optimization showed modest differences from other published high affinity sites. Non-nucleosomal DNA fragments, that fraction of DNA enriched in regions upstream of transcribed sequences (Tazi and Bird, Cell, 60:909-20), were isolated from HEL cells, size fractionated and selected on a GST-GATA-1 affinity column. Retained fragments were eluted, cloned and analyzed by cross competition in EMSAs; results indicated approximately 90% of the cloned fragments preferentially competed for GATA-1. Sequences from 90 isolated clones, while not corresponding to any of the phylogenetically footprinted regions, may represent other, as yet undescribed, loci influenced by this factor. Further analyses of these sequences and comparison with other isolation procedures and application to other DNA binding proteins will be discussed.

N 211 DEVELOPMENTAL DEFECTS IN THE LENS OF HPV-16 E6 OR E7 TRANSGENIC MICE: EVIDENCE FOR TUMOR SUPPRESSOR GENE FUNCTION. Anne E. Griep and Huichin Pan, Department of Anatomy, University of Wisconsin School of Medicine, Madison, WI 53706.

Tumor suppressor gene products such as Rb and p53 are believed to play important roles in regulating cell proliferation and differentiation. During ocular lens development, a specific subset of undifferentiated epithelial cells acquire the capacity to divide, migrate and differentiate. This pattern of growth and differentiation organizes the lens into three spatially separated compartments of quiescent, proliferating and terminally differentiating cells. We have sought to study the function of tumor suppressor gene products in this process in transgenic mice by using the HPV-16 E6 and E7 oncogenes as *trans*-dominant negative effectors of tumor suppressor gene function. Recently, we demonstrated that coexpression of HPV-16 E6 and E7 in the lens leads to an efficient disruption of the normal lens development program. This lens phenotype is characterized by inhibition of fiber cell elongation, induction of abnormal cell proliferation and in certain cases tumor development (J. Virol. 67: 1373-1384). To dissect the contribution of each oncogene to the observed lens defects, we have now generated a series of transgenic mouse lineages expressing individually E6 or E7. Analyses of these mice indicate that E6 and E7 each have distinct effects on differentiating lens cells. Expression of E7 elicits a microphthalmic, cataractous phenotype in the eye, resulting from inhibition of fiber cell differentiation, induction of abnormal cell proliferation, and cell death in the developing lens. However, expression of an E7 mutant which fails to bind to Rb and Rb-like proteins results in a normal lens phenotype. These results suggest that the function of Rb and/or Rb-like proteins is essential in controlling cell proliferation and cell death during lens fiber cell differentiation. Eyes expressing E6 only are not microphthalmic. The E6 lenses do not show evidence for inhibition of fiber cell elongation or for abnormal cell proliferation and cell death. However, adult mice have cataracts, which are characterized at the histological level by retention of cell nuclei in the center of the lens, where enucleation normally occurs. Work is in progress to further characterize the E7-induced cell death and the specific cellular proteins targeted by E7 and to determine if the basis for the effects of E6 on enucleation involve interaction with p53.

N 212 CONFORMATIONAL SHIFTS OF p53 RESTORE DNA BINDING TO SELECT MUTANTS ASSOCIATED WITH HUMAN TUMORS, Thanos D. Halazonetis, Jill Shenk and Jennifer Waterman, Department of Molecular Oncology, The Wistar Institute, Philadelphia, PA 19104

The p53 tumor suppressor protein is mutated in the majority of human tumors. Most of the mutations are single amino acid substitutions, which universally abolish DNA binding activity. We have recently demonstrated that DNA binding of wild-type p53 is conformation-dependent. These studies have now been extended by analysis of oligomerization domain mutants of p53. The conformational shift of wild-type p53 involves the tertiary structure of the sequence-specific DNA binding domain (which we have mapped to residues 90-286) and the quaternary structure of p53 tetramers. However, the stoichiometry of p53 oligomers is not affected by the conformational shift. Wild-type p53 maintains a tetrameric state in both DNA bound and non-bound conformations.

The ability to dissociate the conformational shift of wild-type p53 into tertiary and quaternary structural components, allowed us to modulate the conformation of select p53 mutants and restore to them sequence-specific DNA binding activity to essentially wild-type levels. One of these mutants is the histidine^{273} mutant, which is frequently associated with human tumors. We are currently extending these observations to include the ten most frequent mutants associated with human tumors and to assess, whether in addition to DNA binding, the tumor suppressing activity is restored.

N 213 Developmental Regulation of p110^{RB1} and G_1 Cyclin Expression During Differentiation of the EC Cell Line, P19

Paul A. Hamel and R. M. Gill
Department of Cellular and Molecular Pathology, University of Toronto, Toronto, Ontario M5S 1A8

The embryonic carcinoma cell line (EC), P19 provides a good model system in which to study the mechanisms that control differentiation of specific tissues. In the presence of retinoic acid, P19 cells can be induced to differentiate into a neuronal lineage where neurons, glia and astrocytes are formed. Alternatively, in the presence of DMSO, P19 cells will form actively beating cardiac muscle as well as skeletal and small amounts of smooth muscle. Another interesting feature is that in the undifferentiated state, P19 cells have doubling time of approximately 10 to 12 hours. This short cell cycle is due to a very short G_1 phase: cells begin S within 2 hours following mitosis. Differentiation is accompanied by an initial increase in the length of G_1 and, ultimately, exit from the cell cycle in the case of neurons and cardiac muscle. We have previously demonstrated that expression of the *RB1* gene product, p110^{RB1}, is increased 8 to 10 fold during differentiation into neurons but is only slightly elevated in the muscle-specific pathway. This increase occurs at point in where a lengthened G_1 appears suggesting p110^{RB1} may be involved in the commitment to specific differentiated phenotypes.

Given the extremely short G_1 in the undifferentiated state, we hypothesized that the G_1 cyclins, cyclins C, D1, D2, D3 and E, may be expressed constitutively. Furthermore, recent evidence from a number of labs has suggested that the D cyclins are expressed in a tissue specific manner. Thus, we have begun studying the expression pattern of the G_1 cyclins as well as cyclin A and their associated kinases in undifferentiated P19 cells and RA- and DMSO- induced cells. Our initial results indicate that the D cyclins are expressed at constitutive but relatively low levels in undifferentiated cells. Following induction with RA or DMSO, these cyclins show different patterns of expression in these two differentiation pathways. In the case of cyclin A, strong induction was observed in both the RA-induced neuronal lineage and the DMSO-induced muscle lineage. However, induction was seen late in differentiation suggesting that this induction was occurring in cells other than neurons or cardiac muscle, both of which are in a post mitotic state when cyclin A becomes induced. We are currently identifying the cells specifically expressing the cyclin A and some of the D cyclins as well as the expression pattern of the cyclin dependent kinases.

N 214 THE RB GENE EXPRESSION PREVENTS CELL DEATH IN A BLADDER CARCINOMA CELL LINE. Hashimoto,T.[1], Furuyama, J.[1], Morimura, T.[2], Shindo, H.[3], Tani, E.[3], Tamaoki, T.[4], and Takahashi, R. [5] [1]Dept. Genetics, Hyogo Coll. Med., Nishinomiya, 663, Japan, [2] Dept. Neurosurg., Udano Hosp., 616, Kyoto, Japan, [3]Dept. Neurosurg., Hyogo Coll. Med., Nishinomiya, 663, Japan, [4]Dept. Med. Biochem., Univ. Calgary, Calgary, T2N4N1, Alberta, Canada, [5] Dept. Pathol., Kyoto Univ., Kyoto, 606, Japan

An RB-positive cell line, H-CL2, was established by transfecting an RB expression vector, pBARB, into the RB-negative human bladder carcinoma cell line, HTB9 (Takahashi, R., *et al.* Pro. Natl. Acad. Sci. U.S.A., 88; 5257-5261, 1991). H-CL2 cells showed a longer population-doubling time than HTB9, lack the ability to form colonies in soft agar, and exhibit no tumorigenicity in nude mice. H-CL2 cells also showed serum dependent growth. At confluency, there were no mitotic cells in H-CL2 cultures, whereas mitotic cells were observed in HTB9 cultures. HTB9 cells detached from the plate within several hours after reaching confluency. DNA extraced from floating cells showed DNA ladder formation characteristic of apoptotic cells. At the same time, these cells were dye exclusive when stained with trypan blue, and fragmentation of the nuclei was observed. In contrast, none of these phenomena was observed with H-CL2 at confluency. In HTB9 cells, c-*fos* was expressed transiently after medium change, whereas in H-CL2 cells, it was expressed continually. Expression of c-*myc* in HTB9 was greater than that in H-CL2 when cells were cultured in a low serum medium. HTB9 showed increase in c-*myc* expression followed by DNA synthesis after serum stimulation, whereas no such increase in c-*myc* expression and DNA synthesis was observed in H-CL2 cells. These results suggest that RB protein expression might prevent cell death of HTB9 cells. One possiblity is that the RB protein may down-regulate c-*myc* and other genes leading to suppression of DNA synthesis, cellular growth and apoptotic cell death.

N 215 TRANSCRIPTIONAL REGULATION OF E2F1 DURING THE GROWTH CYCLE, Kuang-Ming Hsiao, Stephanie McMahon and Peggy J. Farnham, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

To further elucidate the signal transduction pathway induced by serum which leads to the activation of genes required for DNA synthesis, we are investigating the mechanism by which E2F1 expression is regulated. The levels of E2F1 mRNA increase at the G1/S-phase boundary. To study the regulatory mechanism of E2F1 expression, we cloned the E2F1 promoter using an oligonucleotide derived from mouse E2F1 cDNA as a probe. Primer extention analysis was performed using RNA from NIH 3T3 cells and a HeLa cell-free system to determine the transcription initiation site. Transcription initiates 118 bases upstream of the translational start site. *In vitro* transcription analysis demonstrated that a 274 bp fragment spanning -176 to +98 of the E2F1 5'-flanking sequences constituted a functional promoter. When this 274 bp fragment was inserted upstream of a luciferase cDNA and transiently transfected into NIH 3T3 cells, we observed that the luciferase activity was low in serum-starved cells and increased approximately 100-fold at the G1/S-phase boundary. The E2F1 promoter activity correlates with E2F1 mRNA expression pattern in the growth cycle. The promoter fragment contained two inverted E2F sites which raised the possibility that E2F may be involved in the regulation of E2F1 expression. We found that expression of mouse E2F1 cDNA stimulates the E2F1 promoter approximately 100-fold in serum-starved cells. We are now analyzing the E2F1 promoter to see if there are any other *cis* elements participating in the regulation of E2F1 expression.

N 216 FUNCTIONAL INTERACTION OF RB WITH A NOVEL MULTIDOMAIN ZINC-FINGER PROTEIN, Shi Huang*, George Steele-Perkins*, Elizabeth Fortunato*#, and Inge Buyse*, *La Jolla Cancer Research Foundation, La Jolla, CA 92037, and #Department of Biology, University of California, San Diego, La Jolla, CA 92037

We have cloned a novel rat nuclear protein of 1706-amino acid, designated RIZ, that contains Rb-binding motifs similar to the conserved cr1 and cr2 regions of several viral oncoproteins. RIZ and Rb interact both in vitro and in vivo. The interaction closely resembles that of SV40 T antigen in both affinity and specificity, and can be similarly disrupted by T and RIZ derived peptides. RIZ interacts specifically with the T/E1A-binding "pocket" domain of Rb that is the major site of naturally occuring mutations. Also like Rb-binding viral oncoproteins which all contain functional zinc fingers, RIZ consists of 8 classical C2-H2 or C2-HC type fingers, and is related to the PRDI-BF1 transcriptional repressor by finger motifs and a novel 100-amino acid homology. Other sequence motifs of RIZ include GTP-binding, Src homology 3 (SH3), and SH3-binding domains. Bacterially expressed RIZ finger motifs bind to DNA in a zinc-dependent fashion. A recombinant RIZ protein containing the GTP-binding domain specifically bind to GTP.

Co-transfection of RIZ with Rb into Rb-defecient SAOS2 cells overrides Rb-mediated growth arrest not through a simple sequestration mechanism. RIZ mRNA of 7.2 kb is preferentially expressed in both adult and embryonic rat neuroendocrine tissues including brain and pituitary, which correlates with the spatial and temporal pattern of phenotypes associated with Rb mutations. These findings provide evidence for a physiological RIZ interaction with Rb, and suggest that RIZ may play an essential role in the Rb mediated tumor suppression pathway.

N 217 p53 INHIBITION OF CELLULAR ENZYMES INVOLVED IN DNA METABOLISM IS RELIEVED BY ITS INTERACTION WITH REPLICATIONPROTEIN A, Ulrich Hübscher[1], Elena Ferrari[1,2], Christian Floth[1] Anthi Georgaki[1], Vladimir N. Podust[1] and Carol Prives[2], [1]Department of Veterinary Biochemistry, University of Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland and [2]Department of Biological Sciences, Columbia University, Sherman Fairchild Center for the Life Sciences, New York, N.Y. 10027, USA

The tumor suppressor protein p53 can exert strong negative regulation on the growth of mammalian cells in culture. We have therefore examined the possibility that p53 modulates the functions of enzymes directly involved in DNA replication and repair. We have shown that intact p53 binds directly to calf thymus single stranded DNA binding replication protein A, RP-A. We have also found that p53 blocks the function of seven DNA helicases including four separate enzymes purified from calf thymus, polyoma large T antigen, simian virus 40 large T antigen and *Escherichia coli* DNA helicase I. This may be related to the fact that p53 possesses the ability to reanneal complementary DNA single strands. Since both wild-type and some mutant forms of p53 protein possess these abilities, these functions are unlikely to be related to the p53 tumor suppressor function. Unexpectedly, we determined that the p53 inhibition of cellular but not viral DNA helicases could be released by RP-A. Additionally, we found that p53 is able to partially inhibit DNA polymerases δ and ε holoenzymes on singly primed M13 DNA. However this inhibition was only observed when *Escherichia coli* SSB, but not when calf thymus RP-A are used in the *in vitro* replication assay. Our data thus show that p53 exerts an inhibitory effect on several enzymes involved in general DNA replication and DNA repair. The p53-RP-A interaction may function to protect replication enzymes from inhibition by p53 under certain physiological conditions.

N 218 A PRB-BINDING PROTEIN (RBP60A) FORMS A HETEROMERIC COMPLEX WITH SP1 AND ACTIVATES TRANSCRIPTION THROUGH THE SP1-SITE, Min-Ho Lee, Srilata Bagchi, Subir Kumar Ray, Shigetoshi Mori, and Pradip Raychaudhuri. Department of Biochemistry (M/C 536), University of Illinois at Chicago, Chicago, IL 60612

We have isolated a cDNA clone for the gene encoding the retinoblastoma-binding protein, RBP60A. The 60kD polypeptide encoded by the cDNA contains a proline-arginine-rich sequence motif that is both necessary and sufficient for binding to the retinoblastoma protein (pRB). The binding site of RBP60A on pRB overlaps with the binding site of the DNA virus oncoproteins. A synthetic peptide coresponding to the adenovirus E1A protein competes with RBP60A for binding to pRB. RBP60A is not a DNA binding protein; however, it is a potent activator of transcription. RBP60A activates transcription from a variety of chimeric promoter constructs that contains Sp1 binding site(s). The mutants of RBP60A that do not interact with Sp1 are also unable to stimulate transcription through the Sp1 site. We speculate that RBP60A activates transcription by bridging the interaction of Sp1 with the 'basal' transcription factors (for example, TBP, TAFs, and TFIIB). We also suggest that the retinoblastoma tumor suppressor protein controls Sp1-dependent transcription through RBP60A.

N 219 CELLULAR PROTEINS MODULATE NONSPECIFIC AND SEQUENCE-SPECIFIC DNA BINDING OF A CONFORMATIONAL DOMAIN OF P53, Steve A. Maxwell, Ramaprasad Srinivasan, and Jack A. Roth, Department of Thoracic and Cardiovascular Surgery, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

Many mutations observed in p53 in human cancers have been found to cluster between residues 100 and 300. This region of p53 can be referred to as a "conformational" domain, since mutations result in alterations in tertiary structure perturbing DNA binding of the protein. The conformational domain also interacts with the SV40 large T-antigen protein, which also disrupts the DNA binding activity of p53. Cellular proteins thus might bind to the conformational domain to regulate the DNA binding activity of p53. We used a hybrid protein comprised of the conformational domain of p53 fused to protein A to probe for cellular binding proteins. Five cellular proteins ranging in sizes from 35K to 90K Mr associated with the wild-type p53 hybrid protein in detergent extracts of non-small cell lung carcinoma cells. These proteins bound to the p53 sequence of the hybrid protein based upon two lines of evidence. First, p53 hybrid proteins containing conserved mutations at residues 175 and 273 bound the cellular proteins less efficently than wild-type p53 hybrid protein. Second, T-antigen competed with the cellular proteins for binding to wild-type p53 hybrid protein. The p53 hybrid protein also exhibited both non-specific binding to calf thymus DNA and sequence-specific binding to an oligonucleotide containing a p53 consensus DNA binding site (p53CON). DNA binding of the hybrid protein was determined to be specific to p53 sequences as based upon the inability of protein A to bind DNA, lack of binding of p53 hybrid protein to mutant p53CON oligonucleotide, and by the sensitivity of DNA binding to the metal chelator 1,10-phenanthroline. The cellular proteins binding to the conformational domain of p53 enhanced DNA binding by 2 to 3-fold. Since the cellular proteins binding to the conformational domain of p53 are differentially expressed in active- and slow-growing cultures and in different NSCLC cell types, they may regulate the interaction of p53 with either the promoters of growth regulatory genes and/or DNA at replication origins.

N 220 THE RETINOBLASTOMA PROTEIN RB CAN DISPLACE THE TATA-BOX BINDING PROTEIN FROM THE CELLULAR TRANSCRIPTION FACTOR E2F-1. Angela Pearson and Jack Greenblatt, Banting and Best Department of Medical Research and the Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, CANADA M5G 1L6

The cellular transcription factor E2F-1 can function as a transcriptional activator when bound to DNA. The activation domain of E2F-1 is localized to its carboxy terminus, is rich in acidic residues, and can function in both yeast and mammalian cells. We have demonstrated using protein affinity chromatography that the activation domain of E2F-1, like those of acidic activators such as VP16 and human p53, interacts directly with the TATA-box binding protein (TBP) of both yeast and mammalian origin. E2F-1 also interacts with the retinoblastoma protein (Rb), and interaction with Rb negatively regulates the transcriptional activity of E2F-1. It has previously been shown that the binding site for Rb is localized to sixteen amino acids within the activation domain of E2F-1 and we have shown that the binding site for TBP overlaps with that for Rb. We have demonstrated using recombinant Rb that a part of Rb sufficient for binding to E2F-1 is able to displace bound TBP from immobilized E2F-1. A mutant version of Rb deficient in binding to E2F-1 is similarly deficient in its ability to displace bound TBP. These results suggest that Rb inhibits transactivation by E2F-1 by preventing the interaction of the activation domain of E2F-1 with TBP.

N 221 WILD-TYPE P53 BINDS TO AN ORIGIN OF BIDIRECTIONAL REPLICATION OF MOUSE rDNA REPEATS

Jürgen Pesch, Eric Gögel, Friederike Stolzenburg and Friedrich Grummt, Institut für Biochemie, Biozentrum, Universität Würzburg, D-97074 Würzburg, Germany

A chromosomal origin of DNA replication has been mapped in the nontranscribed spacer (NTS) of mouse rDNA using two different approaches. The first method based on the screening for amplification promoting sequences (APS) in the 44 kb long rDNA repeat units. Two independent APSs were detected, muNTS1 and muNTS2; approximately 4.1-5.0 kb upstream the transcription initiation site in the NTS region of murine rDNA. As a second origin mapping method we used the PCR technique described by Vassilev and Johnson (NAR 17, 7693, 1989). The data clearly demonstrated the activity of an origin of bidirectional replication in vivo in a region between -0.2 and -5.0 kb upstream the transcription initiation site in the NTS region of murine rDNA. Data base analysis revealed sequence specific binding sites for the tumor suppressor protein p53 in the amplification promoting element muNTS2 of the initiation zone for DNA replication in the mouse rDNA repeats. South-Western experiments as well as immunoprecipitation assays according to McKay and gel retardation assays demonstrated that wild-type p53 binds sequence specifically to the mouse muNTS2 element, while no binding was demonstrated using mutant p53. Correlations between the tumor suppressor protein p53 and the amplification promoting activities of the origin of bidirectional replication in mouse rDNA repeats will be discussed.

N 222 MUTANT p53 INHIBITS TGFß TYPE II RECEPTOR mRNA EXPRESSION AND TGFß-MEDIATED REPRESSION OF HUMAN PAPILLOMAVIRUS TYPE 16 p97 PROMOTER. Michael Reiss and Teresita Muñoz-Antonia. Section of Medical Oncology, Yale University School of Medicine, 333 Cedar Str., New Haven, CT 06510, USA.

We have recently shown that murine keratinocytes transfected with an activated mutant p53 ($p53^{mu}$, 132-Phe) cDNA became partially resistant to the antiproliferative effect of Transforming Growth Factor-ß (TGFß)[1].

In order to determine the mechanism whereby $p53^{mu}$ interferes with TGFß action, we investigated the expression of TGFß type II receptor (TßR-II) mRNA. For these experiments we used mouse tracheal epithelial M2 cells, which had been stably transfected with a temperature-sensitive $p53^{mu}$ (135-Val) cDNA (Mp53.2B cells)[2]. This $p53^{135\text{-}Val}$ protein assumes a wild type conformation at 32°C and a mutant conformation at 37°C.

We analyzed the effect of temperature (and thus of the mutant $p53^{135\text{-}Val}$) on the level of expression of TßR-II mRNA, using an RNAse protection assay. At 32°C, Mp53.2B cells (wild type p53) and M2neo control cells, which only express endogenous wild type p53, expressed equal levels of TßR-II mRNA. However, at 37°C, when the transfected $p53^{135\text{-}Val}$ protein assumes the mutant conformation, TßR-II mRNA levels in Mp53.2B cells were strongly decreased compared to those in M2neo cells.

To determine whether repression of TßR-II receptors resulted in loss of responsiveness of TGFß-regulated genes, we examined the effect of temperature on the repression of the p97 promoter of the human papillomavirus type 16 E6 and E7 genes by TGFß. A p97-luciferase construct (p97-LUC) was used as a reporter gene. Treatment of Mp53.2B cells with TGFß at 32°C suppressed the activity of p97-LUC by greater than 90%. In contrast, p97-LUC expression was not inhibited in cells treated with TGFß at 37°C, when $p53^{135\text{-}Val}$ assumes a mutant conformation. In the control cell line, M2neo (wild type p53), the activity of p97-LUC was inhibited equally strongly by TGFß at both temperatures.

These results indicate that mutant forms of p53 may interfere with TGFß's antiproliferative activity by repressing TßR-II expression, which, in turn, results in loss of repression of TGFß-regulated genes.

[1] Reiss, M. et al. Cancer Res. 1993. 53:899
[2] Gubler, M.L. et al. Proc. Am. Assoc. Cancer Res. 1992, 33:367

N 223 COMPLEX FORMATION ON THE E2F SITE IS MODULATED DURING INDUCED ERYTHROID DIFFERENTIATION, Victoria M. Richon, Hiroaki Kiyokawa, Gisela Perez, Richard A. Rifkind and Paul A. Marks, DeWitt Wallace Research Laboratory, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021

E2F elements are present in the promoter regions of several genes (including c-myb) involved in cell cycle regulation and differentiation. We have studied the molecular basis of terminal cell differentiation using hexamethylene bisacetamide (HMBA) induced erythroleukemia cells (MELC) as a model. We previously found that c-myb expression is down-regulated during induced MELC differentiation. HMBA must be present during G_1/early S to induce subsequent differentiation which is first detected in a proportion of cells (~15%) during the next G_1 which is prolonged. Cells resume cycling for about two to five more generations and arrest in G_1/G_0 with >90% differentiated cells. During the initial prolonged G_1 there is an increase in underphosphorylated retinoblastoma (pRB), decrease in cdk4 and increase cyclin D3 proteins. Underphosphorylated pRB indirectly regulates the transcription of genes, at least in part, by binding to and negatively regulating the transcription factor E2F. We determined the effect of HMBA-induced differentiation on proteins bound to the E2F site of the adenovirus E2a promoter using gel retardation assays. Three complexes were detected in nuclear extracts from uninduced cultures. The complex with the fastest mobility is a free E2F complex as determined by its ability to interact with GST-pRB added to the binding reaction. Following induction of differentiation, the free E2F complex was not detected and there was one predominant complex of slower mobility detected. A complex containing pRB and E2F forms following HMBA-induction of differentiation. All complexes detected were specific since addition of excess unlabeled E2F-site containing competitor oligonucleotide, DRTF 71/50 but not DRTF 62/60 containing a mutated E2F site competed out the detected complexes. An E2F-like site in the promoter region of the c-myb gene also competitively inhibited E2F binding activity. These findings suggest that E2F activity is regulated during induced differentiation of MELC and its negative regulation may lead to induced terminal cell division.

N 224 IDENTIFICATION OF A P53 BINDING SITE IN THE HUMAN RETINOBLASTOMA SUSCEPTIBILITY GENE PROMOTER, Paul D. Robbins[1], Nicole E. Osifchin[1,2], Di Jiang[1], Naoko Ohtani-Fujita[3], Tsuyoshi Fujita[3], Michael Carroza[1] and Toshiyuki Sakai[3], [1]Department of Molecular Genetics and Biochemistry and [2]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261 and [3]Department of Preventive Medicine, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamikyo-ku, Kyoto 602 JAPAN.

p53 is a tumor suppressor gene found to be mutated in a wide variety of human tumors. The encoded p53 protein has properties of a classical transcription factor, but the promoter targets for its regulation are largely unknown. We have investigated the ability of p53 to regulate activity of the human retinoblastoma susceptibility gene (Rb) promoter using a cotransfection assay in CCL-64 and Saos-2 cells. p53 was able to stimulate transcription from the Rb promoter at low input doses of p53 expression plasmid whereas transcription was repressed at high input doses. The stimulatory effect of p53 on Rb promoter activity was localized to a region between 4 and 92 base pairs upstream from the start site of translation, whereas the region controlling repression by p53 was localized to the basal transcriptional region of the promoter between -207 and -185. Gel mobility shift analysis has demonstrated that a p53-containing complex can bind to an oligonucleotide containing Rb promoter sequences between -63 and -88 (Rbp53). Moreover, the preincubation of extracts with SV40 T-antigen inhibited binding of the complex to the Rbp53 sequence. Methylation interference analysis has identified a region with 9 out of 10 base pair homology with a consensus p53 binding site as important for binding of the p53-containing complex. The Rbp53 oligonucleotide is sufficient to confer stimulation by p53 when inserted upstream from a minimal heterologous promoter. The presence of a functional p53 binding site in the human retinoblastoma promoter suggests that the p53 can regulate the level of Rb expression. Further characterization of the Rbp53 binding site and the regulation of the Rb promoter by p53 will be presented.

N 225 SEARCHING FOR THE FUNCTION(S) OF THE p53 SUPPRESSOR GENE IN NORMAL CELLS, Varda Rotter, Ronit Grinstein-Aloni, Dov Schwartz, Barry Alkind, Arnold Simons, Roland Wolkovitch, Pierre Besserman, Ahuva Kapon and Naomi Goldfinger. Department of Cell Biology, The Weizmann Institute, Rehovot, ISRAEL

Inactivation of the p53 tumor suppressor gene plays a major role in malignant transformation. Our *in vitro* experiments have suggested that p53 is involved in cell differentiation in the B-cell lineage. When constitutive expression of wild-type p53 was instituted in a pre-B p53 nonproducer cell line, the cells advanced in their stage of differentiation. Under *in vivo* conditions, expression of wild-type p53 in those p53-nonproducer pre-B cells, caused them to develop smaller size tumors at a lower frequency than following injection of the parental p53 nonproducers. The conclusion that p53 plays a role in normal development and differentiation *in vivo* is substantiated by our studies with p53-promoter-CAT hybrid transgenic mice. We found that p53 expression in adult mice is significantly high in the testes, where it might play a role in the process of sperm cell differentiation and maturation. Analysis of DNA content coupled with p53 expression showed that p53 is expressed in a temporally regulated fashion during spermatogenesis and is confined to the pachytene stage of the meiotic cell cycle. We found that the endogenous levels of p53 mRNA and protein of the p53-promoter CAT transgenic mice were reduced compared to the non-transgenic control mice. The various p53-promoter-CAT transgenic mice exhibited in their testes multinucleated giant cells, a degenerative syndrome resulting from the inability of the tetraploid primary spermatocytes to complete meiotic division. The giant cell degenerative syndrome was also observed in some genetic strains of p53 null homozygotic mice. In view of the hypothesis that p53 plays a role in DNA repair mechanisms, it is tempting to speculate that the physiological function of p53 that is specifically expressed in the meiotic pachytene phase of spermatogenesis is to allow adequate time for the DNA reshuffling and repair events which occur at this phase to be properly completed. Primary spermatocytes which have reduced p53 levels are probably impaired with respect to their DNA repair, thus leading to the development of genetically defective giant cells that do not reach maturation.

N 226 CONTRASTING PATTERNS OF RETINOBLASTOMA PROTEIN EXPRESSION IN MOUSE EMBRYONIC STEM CELLS AND EMBRYONIC FIBROBLASTS.

Pierre Savatier*, Laszlo Szekely&, Klas G. Wiman& and Jacques Samarut*. *Ecole Normale Supérieure de Lyon, UMR 49 CNRS/INRA, 46 allée d'Italie, 69364 Lyon Cedex 07, France. & Department of Tumor Biology, Karolinska Institute, S-104-01 Stockholm, Sweden.

The expression of the retinoblastoma susceptibility (RB-1) gene was investigated in highly proliferating mouse embryonic stem (ES) cells and in slowly proliferating mouse embryonic fibroblasts. The RB protein was expressed at the same level in these two cell types. However, almost exclusively hyperphosphorylated RB was detected in exponentially-growing ES cells (80% of the cells in the S and G2/M phases of the cell cycle). Embryonic fibroblasts and embryonic stem cells were synchronised by colcemid block followed by mitotic shake-off. In embryonic fibroblasts, DNA replication started 10-15 hours after exit from mitosis and RB was transiently dephosphorylated during the G1 phase as previously described. In ES cells, DNA replication started two hours after release from the colcemid block but virtually no hypophosphorylated RB was observed after the release. Instead, there was a marked decrease in the total RB protein level between exit from mitosis and entry into S phase. Absence of hypophosphorylated RB and cell cycle-dependent change in total RB protein level may be relevant to the high proliferation rate and to the tumorigenic nature of mouse embryonic stem cells.

In vitro analyses will be presented that investigate the effects of hypophosphorylated RB overexpression on proliferation and differentiation of ES cells.

N 227 p53 BINDS SINDLE-STRANDED DNA ENDS AND CATALYSES DNA RENATURATION AND STRAND TRANSFER,

Galina Selivanova, Georgy Bakalkin*, Tatjana Yakovleva*, Kristinn P. Magnusson, Laszlo Szekely, Elena Kiseleva#, George Klein, Lars Terenius* and Klas G. Wiman, Departments of Tumor Biology, *Drug Dependence Research, and #Molecular Genetics, Karolinska Institute, S-171 77 Stockholm, Sweden

The p53 tumor supressor protein has previously been shown to bind double stranded (ds) and single stranded (ss) DNA.The ability of p53 to catalyze DNA renaturation and DNA strand transfer was analyzed by incubation of purified p53 protein with various labelled DNA fragments, followed by native PAGE. Both a bacterially expressed wild type p53 protein and a GST-wild type p53 fusion protein catalyzed renaturation of different short (25-462 nt) complementary single-stranded DNA fragments. GST-mutant p53 fusion proteins Glu213, Ile237, and Tyr238, derived from mutant p53 genes of Burkitt lymphomas, failed to catalyze this reaction. In addition, wild type p53 promoted strand transfer between short (36 bp) duplex DNA and complementary single-strand DNA. In contrast to specific DNA binding activity, shown to be activated by PAb421, p53 DNA renaturation and strand transfer activities were inhibited by PAb421, as well as PAb1801. Wild type p53 had significantly higher binding affinity for short (25-40 nt) than for longer (> 450 nt) single-stranded DNA fragments. Moreover, electron microscopy showed that p53 preferentially binds ss DNA ends. This finding suggest that p53 can serve as an intracellular sensor of DNA strand breaks in vivo. The interaction of p53 with ssDNA ends could trigger a conformational change in the protein, leading to activation of DNA repair pathway. Conceivably, the binding of two DNA ends to a p53 oligomer may align DNA fragments and thereby promote DNA renaturation in vitro and, possibly in vivo. Our results raise the possibility that p53 may play a direct role in renaturation and strand transfer reactions required for repair of damaged DNA, including joining of complementary single-strand DNA ends.

N 228 TGFß INHIBITS BRANCHING MORPHOGENESIS AND N-*MYC* EXPRESSION IN LUNG BUDS IN ORGAN CULTURE,

Rosa Serra and Harold L. Moses, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN37232

Lung buds isolated from 11.5d p.c. mouse embryos survive and undergo branching morphogenesis in culture. This organ culture system was used to examine the role of TGFß in lung branching morphogenesis. TGFß was shown to reversibly inhibit branching morphogenesis in lung bud cultures. Inhibition of branching morphogenesis was dependent on the concentration of TGFß and was apparent by 18 to 24h of TGFß treatment. N-*myc* was previously shown to be expressed during embryonic development in epithelial cells involved in branching morphogenesis; c-*myc* expression has been localized to the stroma during this process. Other groups have shown that mice with both N-*myc* genes deleted have defects in lung development. N-*myc* expression was detected by *in situ* hybridization of whole mount and sectioned tissue in the epithelial cells of lung buds in organ culture. TGFß altered the level of N-*myc* in the lung organ cultures. TGFß was shown to inhibit the steady-state level of N-myc RNA at 14 and 48h of treatment as measured by northern blot hybridization. Inhibition of N-*myc* occured prior to the observed changes in morphology. These data suggest that TGFß may inhibit branching morphogenesis by inhibiting expression N-*myc* RNA. Expression of c-*myc* was also examined. c-*myc* RNA levels were inhibited by less than two-fold as measured by northern blot hybridization. *In situ* hybridization of whole mount and sectioned tissue showed that c-*myc* expression was localized to the stroma of untreated and TGFß treated lung buds. The effect of TGFß on lung buds isolated from homozygous RB knock out embryos was also examined. TGFß inhibited branching morphogenesis in the RB-deleted lung buds suggesting that Rb is not necessary for this response. Regulation of N-*myc* in Rb-deleted lung buds is currently under investigation.

N 229 **RAR β2 Suppresses the Growth of Hela Cells**

S P Si, H C Tsou, X Lee, R Buchsbaum and M Peacocke, Departments of Dermatology and Medicine, New England Medical Center and Tufts University School of Medicine. Retinoic acid (RA) and its derivatives are well known to inhibit the growth of a variety of normal and tumor cells. The mechanisms responsible for this, however, are poorly understood. The recent cloning of two distinct but related families of nuclear receptors for retinoids (RARs and RXRs) have provided insight into how RA may exert at least some of its effects. RAR β2 is located on chromosome 3p24, a site suggested to contain a tumor suppressor gene (TS). In order to test the hypothesis that RAR β2 could function as a TS, we generated transfected a RAR β2 construct driven by an RSV promoter into Hela cells, and selected in G418. Southern blotting studies of DNA from these cell lines demonstrated an appropriately sized restriction fragment, and Northern blotting showed both a transfected as well an endogenous transcript. Studies of cell growth demonstrated 50% reduction of cell growth in the transfected cell lines when compared to normal. This growth inhibition was reduced another 20% by 0.1 μM RA, where no effect was seen on the parent cell strains. Anchorage independent growth was also markedly reduced by the presence of the transfected RAR β2, and further reduced by 0.1 μM RA. These data suggest that in certain situations, RAR β2 can inhibit the growth and tumorigenicty of human tumors.

N 230 E1A-INDUCED DIFFERENTIATION OF EMBRYONAL CARCINOMA IS DEPENDENT ON THE BINDING OF p300, Slack R.S., Craig J., Costa S. and McBurney, M.W., University of Ottawa, Department of Medicine, Ottawa, Ontario K1H 8M5

The adenovirus E1A protein is capable of immortalizing primary cell lines presumably by binding a group of cellular proteins thought to control cell growth. Studies with F9 embryonal carcinoma (EC) have indicated that E1A dramatically modified the phenotype of EC cells. Weigel et al. (Proc.Natl.Acad.Sci.(1990)87:9878) have reported that E1A is toxic to EC cells and no viable transformants could be obtained. In contrast, Montano and Lane (Mol.Cell Biol.(1987)7:1782-1790) have shown that EC cells transfected with E1A remained viable and were induced to differentiate to endoderm. We transfected P19 EC cells with both the 12S and 13S E1A and found results consistent with both reports. No clones of proliferating EC cells expressing E1A could be isolated. P19 EC cells transfected with E1A either undergo complete growth arrest or endodermal differentiation. The surviving P19 derived cells expressing E1A are characterized by a marked loss of cell surface embryonic antigen (SSEA-1) expression accompanied by an enhancement of cytokeratin 55 levels. These stable endodermal cell lines continue to proliferate and can be passaged indefinitely. No further change in phenotype could be affected by treatment with either retinoic acid or dimethylsulfoxide. As E1A is known to bind several cellular proteins regulating cell growth, we sought to determine the mechanism by which this oncoprotein induces growth arrest or differentiation in P19 EC cells. The 13 and 12S E1A were equally efficient in inducing the phenotypic change. Colony assays performed on a series of E1A deletion mutants indicated that an intact CR1 was required. Immunoprecipitations with antibodies against E1A and p300 indicate that p300 binding is essential for alteration of the EC cell phenotype and the subsequent endodermal differentiation. Immunoprecipitation patterns obtained from exon 1 deletion mutants unable to induce differentiation differed from wildtype E1A only in their ability to bind p300. Stable transformants carrying these deletion mutants expressed high levels of the cell surface embryonic antigen, could be maintained indefinitely and were identical to cells transfected with vector only. These studies demonstrate that E1A-induced differentiation of EC cells is dependent on the binding of p300.

N 231 DOES E2F1 OR DP-1 PLAY A ROLE IN REGULATED TRANSCRIPTION FROM THE DHFR PROMOTER? Jill E. Slansky and Peggy J. Farnham, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

One goal in our lab is to understand the transcriptional regulation from the mouse DHFR promoter as cells enter S phase of the growth cycle. Similar to other genes which encode enzymes involved in nucleotide biosynthesis, the levels of DHFR RNA increase late in G1. Using a serum starvation and stimulation assay of NIH 3T3 cells, we have previously observed that the E2F binding sites at the transcription initiation site are necessary for the increase in DHFR promoter activity at the G1/S-phase boundary. A family of transcription factors bind to E2F elements (consensus site is TTTSSCGC, S = G or C). To date, two such proteins, E2F1 and DP-1, have been cloned from mouse cells. The mRNA levels of E2F1 correlate with the mRNA levels of DHFR. The mRNA levels of DP1, however, do not dramatically change during the growth cycle. One model is that E2F1 is the regulated partner and DP-1 is the constitutive partner in a heterodimer complex. To determine which, if either, of these two proteins may be responsible for the increased expression of DHFR at the G1/S-phase boundary, we are targeting the messenger RNAs of these proteins with specific hammerhead ribozymes and antisense RNAs. Preliminary results indicate that increasing concentrations of ribozyme specific to E2F1 increase activity from the DHFR promoter in serum-starved cells. These results are consistent with increased activity from the DHFR promoter with mutated E2F sites. In contrast, increasing concentrations of ribozyme specific to DP1 decrease activity from the DHFR promoter in serum-starved cells. Currently, the effects of these ribozymes are being examined in serum-stimulated cells.

N 232 TGFβ EFFECTS ON CELL CYCLE PROGRESSION OF NORMAL AND IMMORTAL HUMAN MAMMARY EPITHELIAL CELLS IN CULTURE, Martha Stampfer, David Alexander, Junko Hosoda, Chin-Huei Pan, and Paul Yaswen, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720

The effects of TGFβ on growth control and specialized functions have been examined and compared in normal finite lifespan human mammary epithelial cell (HMEC) strains and two immortally transformed cell lines, 184B5 and 184A1, derived from normal HMEC specimen 184 following in vitro exposure to benzo(a)pyrene. Normal HMEC are all growth inhibited by TGFβ, however younger cells in vitro may undergo several population doublings before cessation of growth. In contrast, both immortal cell lines can give rise to populations which maintain growth indefinitely in the presence of TGFβ, as well as populations which are largely growth inhibited. All of these HMEC can respond to TGFβ with increased synthesis and secretion of extracellular matrix associated proteins. In order to specifically examine the pathway of TGFβ induced growth inhibition, the effect of TGFβ on cell cycle associated genes and proteins (cyclins, kinases, early response genes, Rb) has been examined in these cell types with widely varying growth responses to TGFβ. HMEC were placed in a Go growth arrest by blockage of EGF receptor signal transduction, and then allowed to synchronously enter the cell cycle by re-exposure to EGF, with and without TGFβ. The level of Rb phosphorylation correlated closely with the extent of entry into S phase; there was no correlation between levels of myc and extent of TGFβ induced growth inhibition. TGFβ did not effect the initial burst of early response gene expression seen upon exit from Go. We are currently examining the relationship between TGFβ inhibition and cyclin expression/kinase activities.

N 233 SUPPRESSION OF THE HUMAN MDR1 DOWNSTREAM PROMOTER BY WILD-TYPE AND MUTANT HUMAN P53, Bryan E. Strauss[1] and Martin Haas[1,2,3], [1]Department of Molecular Pathology, [2]Department of Biology, [3]Cancer Center, University of California, San Diego, La Jolla, CA 92093-0063.

The mdr1 downstream promoter was amplified by PCR from normal human placenta DNA and cloned into a luciferase reporter vector, yielding pMDR-lux. A truncated construct spanning a 125 bp (Bsm1-Sst1) mdr1-promoter fragment driving the reporter gene, pΔΔMDR-lux, was also made. These constructs were used in cotransfection assays in BHK cells with expression vectors for human wild-type p53 , mutant p53 (175H, 213Q, 234H, 248Q), or both, as well as with an internal control construct that is not affected by p53. Wild-type p53 or each mutant p53 suppressed transcription from the mdr1 downstream promoter in a dose-dependent manner. Up to 85% inhibition was recorded. Cotransfection of both wild-type p53 and mutant p53 together yielded results of an additive nature. In contrast to these results, previous reports indicated that wild-type p53 suppresses expression, while mutant activates transcription from the mdr1 promoter (Chin et al, Science, 255:459, Zastawny et al, Oncogene, 8:1529). Functional expression of the mdr1 product is being studied by FACS analysis on drug resistant/sensitive human cells transfected with either wild-type or mutant p53 expression constructs.

In a control experiment, cotransfection of wild-type p53 and $PG_{13}CAT$, a reiterated p53-responsive motif, resulted in its activation, whereas the mutant constructs (175H, 234H, 248Q) failed to affect this motif, in accordance with published data (Kern et al, Nature Genetics, 1:45) In addition, cotransfection of wild-type p53, mutant p53 and the $PG_{13}CAT$ construct resulted in a dominant negative suppression of the ability of the wild-type p53 to activate the PG element. The 213Q mutant demonstrates the ability to transactivate the $PG_{13}CAT$ construct and acts additively with wild-type p53 suggesting that in this assay this mutant p53 protein behaves as wild-type (Zhang et al, Oncogene, 8:2555).

N 234 CYCLIN D1 EXPRESSION IS REGULATED BY THE RETINOBLASTOMA PROTEIN, Michael Strauss[1,2], Heiko Müller[1,2], Jiri Lukas[2], Andreas Schneider[3], Peter Warthoe[2], Martin Eilers[3] and Jiri Bartek[2] [1]Max-Planck-Gesellschaft, Humboldt Universität, Robert-Rössle-Str. 10, D-13125 Berlin-Buch, Germany,[2]Danish Cancer Society, Division of Cancer Biology, Strandboulevarden 49,7.1, DK-2100 Copenhagen, Denmark, [3]Zentrum für molekulare Biologie, Universität Heidelberg, Im Neuenheimer Feld , D-69120 Heidelberg, Germany

The product of the retinoblastoma susceptibility gene, pRb, acts as a tumor suppressor and loss of its function is involved in the development of various types of cancer. DNA tumor viruses disturb the normal regulation of the cell cycle by inactivating pRb. However, a direct function of pRb in the regulation of the cell cycle has hitherto not been shown. We demonstrate here that the cell-cycle dependent expression of one of the G1-cyclins, cyclin D1, is dependent on the presence of a functional Rb protein. Rb-deficient tumor cell lines as well as cells expressing viral oncoproteins (T antigen of SV40, E1A of adenovirus, E7 of papillomavirus) have low or barely detectable levels of cyclin D1. Expression of cyclin D1, but not of cyclins A and E, is induced by transfection of the Rb gene into Rb-deficient tumor cells. Cotransfection of a reporter gene under the control of the D1 promoter, together with the Rb gene, into Rb-deficient cell lines demonstrates stimulation of the D1 promoter by Rb which parallels the stimulation of endogenous cyclin D1 gene expression. Our finding that pRb stimulates the expression of cyclin D1 suggests the existence of a regulatory loop between pRb and cyclin D1 and extends existing models of tumor suppressor function.

N 235 DIFFERENTIAL REGULATION OF GENE EXPRESSION BY A p53-RESPONSIVE ELEMENT. Rebecca Sundseth and A. Christie King. Division of Molecular Genetics and Microbiology, Wellcome Research Laboratories, Research Triangle Park, NC 27709.

The tumor suppressor protein p53 is a DNA-binding protein that both positively and negatively regulates transcription. Trans-activation by p53 is dependent upon specific DNA recognition, but mechanisms controlling transcriptional repression are unknown. To investigate gene regulation by p53, chimeric templates were constructed having a p53 responsive element from the ribosome gene cluster (RGC) and the proximal regions from either the SV40 early or human MDR1 promoters linked to a luciferase reporter gene. Both reporter plasmids, RGC(+)SV40E-luc and RGC(+)hMDR1-luc, are transactivated by p53 (300 and 23-fold activation, respectively) when cotransfected with a p53 expression plasmid into Saos2 osteosarcoma cells. Control reporter plasmids having a mutated RGC element that no longer bind p53 are unresponsive to p53wt. These data confirm that the RGC element and p53wt function together to activate transcription in Saos2 cells. In contrast, when HepG2 hepatocellular carcinoma cells are cotransfected with the same reporter and expression plasmids, there is a reproducible 3-4-fold repression of promoter activity. These are the first data to demonstrate that a cis-element recognizing p53wt does not respond identically in every cell type. Another difference observed in HepG2 cells is that the RGC element, without coexpression of p53wt, causes a 6 to 20-fold elevation of promoter activity above that found with the parent reporter plasmids or those containing a mutated RGC element. HepG2 contain no detectable p53wt protein, therefore, it is likely that promoter activation resulting from the RGC element is not directly due to p53 but to addtional cellular factors having overlapping DNA-binding specificity with p53.

N 236 **DOWNREGULATION OF D TYPE CYCLINS BY VIRAL ONCOPROTEINS AND TUMOR SUPPRESSOR PROTEINS**

Sun W. Tam, Jerry Shay*, Giulio Draetta and Michele Pagano Mitotix Inc. One Kendall Square, Bldg. 600; Cambridge, MA 02139; *The University of Texas Southwestern Medical Center at Dallas, Dept. of Cell Biology and Neurosciences, 5323 Harry Hines Boulevard, Dallas, TX 75235.

Abnormal expression of D-type cyclin genes has been associated with loss of the normal regulation of cell proliferation in several tumor cells. By comparing the abundance of cyclin D1 and D3 proteins in normal human diploid cells and in tumor cell lines, we found that tumor cells expressing SV40 large T or E1A or E7 oncoproteins had very low levels of D1 and D3 in any phase of the cell cycle. Lack of a functional retinoblastoma protein was found to correlate with low D1 and D3 protein levels, while a nonfunctional p53 alone had no effect. We have confirmed these results by analyzing normal human fibroblasts infected with defective retroviruses expressing the HPV-16 E6 and/or E7 proteins. Presence of E6 alone did not change the protein levels of D1 and D3; in contrast, presence of E7 alone or E6 in combination with E7 caused a reduction in D1 and D3 protein levels. In fibroblasts transfected with a plasmid expressing the SV40 large T antigen, the D1 and D3 protein levels also appeared dramatically downregulated. Microinjection experiments with anti-D1 antibodies showed that cyclin D1 becomes dispensable for the progression through G1 in the cells expressing the viral oncoproteins. We are now studying the biochemical mechanism responsible for the downregulation of cyclin D1 and D3 in these tumors.

N 237 ***QSR1*, a yeast essential gene encodes an AP1 negative regulator homolog,** Thierry Tron, Henry D. Riley, Spyridoula Karamanou, Fred Dick and Bernard L. Trumpower, Department of Biochemistry, Dartmouth Medical School, Hanover, N. H. , 03755

We have cloned a novel yeast gene, *QSR1*, by complementing a mutant, *qsr1-1*, which exhibits an aberrant growth phenotype. The gene encompasses a single open reading frame, capable of encoding a basic protein of 221 amino acids and predicted molecular weight 25,392. The QSR1 protein is 67% identical to a human protein of unknown function, which is differentially expressed both in Wilms' and breast tumors. As genes encoding QM homologs have been cloned from other species including bird, invertebrate and plants, this protein appears highly conserved through evolution. In chicken, the QM homolog binds the protooncogene jun and acts as a negative regulator of the AP1 transcription factor.

The yeast mutant complemented by a wild type *QSR1* allele exhibits a pleiotropic phenotype with altered growth parameters, floculation, cell cycle perturbations and an abnormal cell morphology. As the cells enter stationary phase, the complemented *qsr1* forms filamentous, multibudded cells, in which daughter cells fail to separate from the parent. These results indicate that *QSR1* could be involved late in the cell cycle in order for the yeast to complete cytokinesis. Deletion analysis showed that*QSR1* is essential, whereas sequencing of the mutant allele revealed that the toxicity of the mutant protein is linked to a single amino acid replacement in its carboxy-terminal portion. On the basis of its homology to the QM proteins, we speculate that QSR1 could regulate the activity of yeast trancription factors, like GCN4 and YAP1. The screening procedure by which *qsr1-1* was isolated suggests that there is a previously unrecognized relationship between a regulatory subunit of the mitochondrial cytochrome bc_1 complex and cell growth on both fermentable and nonfermentable carbon sources.

N 238 SERINE MUTANTS OF HUMAN P53 IN THE ACTIVATION DOMAIN ARE WILD-TYPE FOR TRANSFORMATION SUPPRESSION AND TRANSACTIVATION FUNCTIONS, Tamar Unger, Martin Scheffner, Carole L. Yee and Peter M. Howley., Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, Maryland 20892

Several serine residues at the amino and carboxy terminus of p53 have been identified as sites of phosphorylation by a number of cellular kinases. We have focused on potential phosphorylation sites at the N-terminal region, where the transcriptional activation domain has been mapped. With the notion that the transactivation function of p53 is important for the transformation suppression of p53 (Unger et al., 1993 Mol. Cell. Biol., 13, 5186-5194), we tested whether mutations of the different Ser residues at the activation domain would affect the function of p53 to act as a tumor suppressor protein. For this purpose we mutated systematically individual Ser residues at positions 6, 9, 15, 20, 33 and 37 either to Ala or to Asp residues as well as all of the above Ser residues to Ala or to Asp. The Ser mutants were examined for their abilities to function in transactivation and cell transformation. The different Ser mutants were transfected into the human lung cancer cell line H358, which does not express p53 and tested for their ability to transactivte a CAT reporter plasmid containing 6 copies of the p53 binding sequence upstream of the reporter gene. All of the different individual Ser mutants as well as all of them either mutated to Ala or to Asp could transactivate the reporter plasmid to a similar levels as wt p53. These Ser mutants could also suppress transformation of a mutant ras gene and adenovirus E1A when transfected into primary rat embryo fibroblasts. Experiments analyzing the interaction of p53 with other cellular proteins with these Ser mutants are in progress.

N 239 THE ROLE OF WT1 IN THE DIFFERENTIATION OF TRANSFORMED HUMAN FOETAL KIDNEY CELLS, Joanne E. Watson and Keith W. Brown. Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, U.K.

The WT1 gene codes for a tumour suppressor gene involved in the development of the kidney, specifically the transition of undifferentiated mesenchymal cells to epithelial cells.

Previous work involving studies on the effects of chemicals including Retinoic acid and dibutyryl cAMP on virally transformed human embryonic kidney cell lines found that 12-O-tetradecanoyl phorbol-13-acetate (TPA) appeared to induce the cell line 293 to flatten out and lose the embryonic marker N-CAM. They were also seen to start producing cytokeratins, a marker of epithelial differentiation.

This result has now been followed up by a series of experiments including serum stimulation to investigate any changes in WT1 expression during this apparent induction of differentiation. To date it has been verified that TPA does induce 293 cells to loose N-CAM expression and to increase cytokeratin expression. Northern blotting, however, has not yet shown any changes in WT1 expression during this transition.

It has also been noted that the treatment of 293 cells with TPA resulted in an increase in the number of unattached cells floating in the medium compared to untreated cells. The DNA from these cells showed the characteristic laddering seen in cells undergoing apoptosis.

The WT1 gene has now been cloned into the inducible expression vector pMEP4, both in the sense and antisense orientations. These constructs have been transfected into 293 cells. Future work will include investigating the effects of transient expression of sense and antisense WT1 transcripts in these cells.

Thus it appears that TPA has two effects on 293 cells; it induces some cells to undergo some form of differentiation, and others to undergo apoptosis.

N 240 THE RETINOBLASTOMA PROTEIN ASSOCIATES WITH THE SEQUENCE SPECIFIC SINGLE-STRANDED DNA BINDING PROTEIN YB-1, Kevin R. Webster, Janet G. Mulheron, Barri S. Wautlet, And Kevin G. Coleman, Department of Molecular Genetics and Cell Biology, Bristol-Myers Squibb Pharmaceutical Research Inst., Princeton, NJ 08543

The retinoblastoma protein (pRb) is an 110 kDa phosphoprotein which is a suppressor of tumor cell growth. pRb has been shown to interact with a number of cellular factors which may contribute to its growth regulatory activities. We have demonstrated that pRb associates with YB-1 protein, a potential regulator of DNA replication. YB-1 protein is a sequence specific, single-stranded DNA (ss-DNA) binding protein which recognizes CCN direct repeats within the c-myc, EGF receptor, and c-fos promoters. YB-1 associates with both the initiators of DNA replication and members of the cyclin dependent kinase (cdk) family. The YB-1 associated kinase activities are cell cycle dependent, with peak activity occurring at the G1/S phase transition. We have shown that both, a GST-YB1 fusion protein can precipitate cellular pRb and a GST-Rb fusion protein can precipitate cellular YB-1 protein. In addition, we have determined that sequences within exon 21 and the carboxy-terminal domain (amino acids 816-928) of pRb are essential for YB-1 protein binding. Furthermore, coimmunoprecipitation of pRb with YB-1 antibody is cell cycle dependent. These interactions implicate pRb in the regulation of DNA replication and offer additional evidence that YB-1 is a regulator of the G1/S phase transition of the cell cycle.

N 241 THE RETINOBLASTOMA PROTEIN HAS TRANSCRIPTIONAL REPRESSOR ACTIVITY THAT IS INDEPENDENT OF ITS INTERACTION WITH E2F, Steven J. Weintraub, K.N.B. Chow, and D. Dean, Department of Internal Medicine, Washington University Medical Center, Saint Louis, Missouri 63110

The retinoblastoma protein (Rb) regulates progression through the cell cycle at least in part through its cell cycle–dependent interaction with the *trans*–activating protein E2F. Binding of Rb to E2F is controlled by the phosphorylation/dephosphorylation cycle of Rb that occurs with progression through the cell cycle. The dephosphorylated form of Rb binds to the transactivating domain of E2F and it had been thought that, when bound, Rb simply blocks *trans*–activation by E2F. However, we recently found that in the presence of Rb, E2F sites switch from transcriptional activators to silencers (Weintraub, et al., *Nature* 358, 259–261)—suggesting that interaction of Rb with E2F does not simply inactivate E2F, but results in the formation of an active transcriptional repressor complex.

To further understand the mechanism of transcriptional repression mediated by E2F sites in the presence of Rb, we have constructed vectors that express chimeric proteins in which either full length Rb or isolated regions of Rb are linked to the DNA binding domain of the yeast transcription factor Gal4, facilitating the tethering of Rb and specific regions of Rb to the promoter in an E2F independent fashion. Using these constructs we found that: 1. Rb has transcriptional repressor activity that is independent of its interaction with E2F. 2. Distinguishable domains of Rb are required for binding E2F and for repression.

N 242 TRANSCRIPTIONAL CONTROL OF THE RB1 PROMOTER, Laura L. Whitaker and Marc F. Hansen, Department of Molecular Genetics, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

The retinoblastoma susceptibility (RB1) gene product has been shown to play a role in cell cycle regulation, specifically at the G1/S boundary. This protein is highly stable, with a half-life greater than 20 hours. RB1 has been described as a ubiquitously expressed tumor suppressor gene, however, its expression has now been shown to be variable within different tissues, with the highest levels of expression being found in differentiating cells. Given the stability of the protein, this suggests that the control of the variable expression must be at the transcriptional level. To address this question, we examined the region upstream of the transcription initiation site for sequences bound by cellular trans-acting factors using gel shift assays and DNase footprinting. Gel shift assays of several of the fragments have demonstrated specific band shifts. Comparison of the shifted sequences to over 2100 known transcription factor recognition sites using the GCG Transcription Factor Database failed to show candidates for the binding proteins responsible for these gel shifts, suggesting that this variation in expression in response to differentiation is likely to be due to the binding of novel transcription factors to the RB1 promoter.

N 243 FREQUENT p53 ABERRATIONS AND ABSENCE OF MDM2 AMPLIFICATION IN POLYOMA VIRUS-INDUCED TUMORS, Klas G. Wiman, Wang Qian, Kristinn P. Magnusson, Elena Kashuba, Ekaterina Pokrovskaia and George Klein, Department of Tumor Biology, Karolinska Institute, 171 77 Stockholm, Sweden

Unlike the SV40 large T antigen that can bind both the retinoblastoma (RB) and p53 proteins, the polyoma virus large T antigen forms a complex with RB but not with p53. If p53 inactivation is required for the development of polyoma virus-induced tumors, one would expect p53 mutation and/or deletion in all these tumors. We therefore examined the p53 status of eight polyoma virus-induced tumors using immunoprecipitation with monoclonal antibodies specific for wild type p53 (PAb246), mutant p53 (PAb240), and wild type as well as mutant p53 (PAb421), and immunostaining with PAb421. Two tumors were completely p53 negative, and two tumors expressed mutant (PAb240+) p53. In the SEWA tumor, DNA sequencing revealed a splice donor site mutation that resulted in 5 extra amino acid codons at the end of exon 7 in the spliced mRNA. Three of the remaining 4 tumors expressed low levels of wild type p53, as shown by immunoprecipitation and DNA sequencing of exons 4 through 9. One tumor, however, expressed elevated levels of apparently wild type p53 by the same criteria. Moreover, Southern blot analysis did not reveal amplification of the MDM2 gene in any of the tumors. Thus, our data demonstrate that inactivation of p53 is frequently but not invariably involved in the genesis of polyoma virus-induced tumors. In order to assess whether p53 function remains intact in the tumors that express wild type p53, we are currently looking to see if p53 accumulates after mitomycin C treatment, and if accumulation of p53 leads to growth arrest or apoptosis.

N 244 A NOVEL GENE THAT INTERACTS WITH BCL-2, Elizabeth Yang, Jennifer Jockel, Jiping Zha and Stanley J. Korsmeyer, Howard Hughes Medical Institute, Washington University School of Medicine, St. Louis, MO 63110

Bcl-2 is a proto-oncogene which functions as a death repressor in a number of programmed cell death pathways. In order to understand the molecular mechanism of Bcl-2 function, we sought to identify its interacting proteins. Using a Gal4-DNA binding domain-Bcl-2 fusion plasmid as bait in the yeast 2-hybrid system, we screened a mouse embryonic fusion library and isolated a novel gene which interacts with Bcl-2. The same gene was also identified when radiolabelled GST-Bcl-2 fusion protein was used to screen an expression library *in vitro*. Two alternatively spliced cDNAs of this gene have been isolated, and the open reading frames share limited regions of homology with Bcl-2. In the yeast 2-hybrid system, this novel protein also interacts with the Bcl-2 homolog Bcl-x, but not Bax. The physiologic consequences of these interactions are being investigated.

Carcinogenesis

N 300 EFFECTS OF C-MYC OVEREXPRESSION ON TGFß1-INDUCED GROWTH ARREST OF BALB/MK CELLS, Mark Alexandrow, Masahiro Kawabata, Mary Aakre and Harold L. Moses, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

Previous research from this laboratory has shown that TGFß1 treatment of mouse keratinocytes (MK) results in the rapid suppression of c-myc mRNA and protein levels. Because TGFß1 can inhibit the growth of MK cells by preventing entry into S-phase, and because c-myc expression is required for normal proliferation of MK cells we wanted to determine if overexpression of c-myc could prevent TGFß1 from inhibiting MK cell growth. Using an estrogen-inducible chimeric c-myc protein consisting of the entire human c-myc protein fused to the hormone binding domain of the human estrogen receptor (mycER) we have found that induction of mycER can block the ability of TGFß1 to inhibit MK cell growth. Control experiments indicate that the ER domain does not contribute to the results we have observed. This effect of the chimera on TGFß1 inhibition was reproducible in rapidly-growing and synchronized populations of MK cells. However, induction of mycER at different times in G1-phase of synchronized cells showed that c-myc overexpression late in G1-phase was ineffective at blocking TGFß1 inhibition although TGFß1 could still inhibit entry into S-phase even when added to the cells just prior to the G1/S transition. We hypothesize that the mechanism of mycER suppression of TGFß1 inhibition is indirect in that mycER induction may be resulting in the overexpression of factors known to be important for cell cycle progression. Temporally, mycER is able to affect the levels of these proteins when it is induced early in G1-phase around the time when endogenous c-myc levels are also at a maximum. Studies to determine which proteins show altered expression due to mycER activation and their importance to TGFß1 signaling are in progress.

N 301 IκBα IS A NOVEL TUMOR SUPRESSOR GENE.

Beauparlant P, Kwan I, Chou P, Bitar R, Hiscott J. Lady Davis Institute for Medical Research. Department of Microbiology, McGill University. 3755 Chemin de la Cote Ste-Catherine . Montreal, Quebec .Canada H3T 1E2.

NF-κB proteins are family of pleiotropic transcription factors present in most cell types in an inactive cytoplasmic form. Cytoplasmic partitioning of latent NF-κB proteins is mediated by the inhibitory IκBproteins that bind to and mask a nuclear translocation signal in the DNA binding NF-κB proteins. Activating agents, such as viruses, cytokines, and bacterial lipopolysaccharide, promote the dissociation of the cytosolic NF-κB/IκB complexes in part by activating cellular kinases that phosphorylate IκB. Several lines of evidence link the NF-κB family of transcription factors to cell growth regulation.

To further understand the association of the NF-κB family with cell growth regulation, the effect of ectopic expression of individual NF-κB genes has been examined in NIH3T3 cells. Initial experiments have focused on the expression of sense and antisense IκBα and IκBγ constructs. Using retroviral mediated transduction via the pMV7 vector, IκBγ was expressed to high levels in NIH3T3 cells without an apparent biological or biochemical phenotype. To date, it has not been possible to isolate cell clones expressing IκBα protein. Interestingly, overexpression of the IκBα anti-sense construct generated high levels of antisense RNA in multiple clones and by several criteria induced cellular transformation of NIH3T3 cells: the cells displayed altered morphology; saturation density in 2% serum was increased 3-4 fold; growth in soft agar occurred at an efficiency of 10%; and most importantly, anti-sense IκBα expressing cells formed tumors in nude mice 16-17 days post injection.

NF-κB specific DNA binding activity in the nucleus of the IkBα-antisense expressing cells was increased 3 fold compared to pMV7- or IκBγ-expressing cells. Similarly, transcription from NF-κB regulated reporter genes was increased in cells expressing the anti-sense IκBα RNA. On the basis of present data, we suggest that IκBα represents a novel tumor suppressor gene that functions in the normal cell to control the cytoplasmic-nuclear partitioning of the NF-κB family of proto-oncogenes. Overexpression of an IκBα antisense may alter the translation or turnover of IκBα, thereby disrupting the NF-κB-IκB autoregulatory loop.

N 302 *p53* ALTERATIONS IN LUNG TUMORS INDUCED IN F344/N RATS, Steven A. Belinsky, Kristen J. Nikula, Fletcher F. Hahn and Deborah S. Swafford, Inhalation Toxicology Research Institute, Albuquerque, NM 87185

The prevalence for alteration of the *p53* gene was examined in primary lung tumors induced in the F344/N rat by beryllium (Be), diesel exhaust (DE), carbon black (CB), and X rays. Alterations within the *p53* gene were assessed by immunohistochemistry (IHC), single strand conformation polymorphism analysis (SSCP), and direct sequencing. None of the adenocarcinomas (AC, n = 62) demonstrated increased p53 protein by IHC. SSCP analysis of exons 4-9 is underway; alterations have been detected in one AC induced by DE and one AC induced by X ray. In contrast, dysfunctional p53 protein was detected in squamous cell carcinomas (SCC) at the following frequencies: three of 18 for X-ray-induced, three of five for DE-induced, and two of three for CB-induced. These data indicate that in the rat, mutation of the *p53* gene in the conserved region is not involved in the development of AC and only infrequently in SCC. Interestingly, SSCP analysis of exons 4-9 of the three X-ray-induced tumors exhibiting dysfunctional p53 protein by IHC failed to detect any mutation. The possibility that the increased binding of p53 protein in the SCC tumors is due to alterations in the *mdm2* gene is currently under investigation. (Research sponsored by the OHER, US DOE under Contract No. DE-AC04-76EV01013 and the Health Effects Institute under Funds-In-Agreement No. DE-FI04-91AL75007 with US DOE.)

N 303 LOSS OF SUPPRESSOR FUNCTIONS INVOLVED IN IMMORTALIZATION AND TUMOR PROGRESSION OF THE HUMAN KERATINOCYTE LINE HACAT, *Petra Boukamp, Wolfgang Peter, Ulrich Pascheberg, Eric J. Stanbridge, Curtis C. Harris, Norbert E. Fusenig,* German Cancer Research Center, 69009 Heidelberg, Germany

Despite considerable increase in non-melanoma skin cancer in man only few models are available to study mechanisms involved in its genesis. We have developed an in vitro model (HaCaT) starting from normal human trunk skin keratinocytes. Immortalization of the HaCaT cells which had occurred spontaneously was correlated with UV-induced mutations in the p53 gene (C to T transition in codon 179 in one allele and CC to TT double base change in the second allele) leading to inactivation of both p53 alleles. In addition the cells had lost one copy of chromosomes 3p, 4p, 9p and the Y chromosome. First single chromosome transfer studies (MMCT) indicate that an extra copy of chromosome 3 can restore senescence in early passage HaCaT cells suggesting that chromosome 3 carries putative senescence gene(s). Tumorigenic conversion could be induced by transfection with the c-Ha-ras oncogene leading to benign and malignant tumorigenic clones (HaCaT-ras). Since both types of clones expressed comparable levels of onco-protein (p21-val12) and also the integration site of the ras oncogene in the cellular genome did not account for a particular phenotype (integration had occurred at random) ras oncogene was obviously prerequisite but by itself insufficient to malignantly transform the HaCaT cells. By MMCT, using chromosomes with stablely integrated ras oncogene as vehicles to transfer the oncogene in a more standardized way (same amount of DNA and same integration site) the tumor phenotype was clearly determined by the genetic make up of the recipient cell. HaCaT cells of later passages carrying only 2 or 3 copies of chromosome 15 became malignant after transfer of the chromosome carrying the ras oncogene while early passages with 4 or more copies remained nontumorigenic or formed benign tumors after s.c. injection in mice. Since chromosomes 15 is also underrepresented in cell lines derived from skin squamous cell carcinomas (SCC) we at present determine whether an extra copy of #15 can suppress tumorigenicity in these cells and therefore may carry a tumorsuppressor gene specific for skin carcinomas.Thus, by using the spontaneously immortalized human skin keratinocyte line HaCaT and transfer of the c-Ha-ras oncogene we could establish a multistep skin carcinogenesis model. Since the observed genetic alterations are also representative for skin SCC lines, these HaCaT cells provide an excellent model to unravel molecular mechanisms involved in the stepwise progression of non-melanoma skin cancer.

N 304 THE TIS1 AND TIS21 PRIMARY RESPONSE GENES ARE CANDIDATE TUMOR PROMOTION SUPPRESSOR GENES, Joan L. Cmarik[1], Harvey Herschman[2], and Nancy H. Colburn[1], [1]Laboratory of Viral Carcinogenesis, NCI-FCRDC, Frederick, MD 21702 and [2]Laboratory of Biomedical and Environmental Sciences, Molecular Biology Institute, and Dept. of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024

We have identified two primary response genes as potential suppressors of the tumor promotion process: TIS1 (nur77/NGFI-B), a member of the glucocorticoid receptor superfamily; and TIS21 (PC3), which encodes a protein of unknown function. These genes were identified by comparing their expression in promotion-sensitive (P+) and promotion-resistant (P-) JB6 murine epidermal cell lines. TIS1 and TIS21 are preferentially expressed in promotion-*resistant* cells in response to tetradecanoyl phorbol acetate (TPA) or epidermal growth factor, both agents which induce the neoplastic transformation of P+ cells. The anti-promoter forskolin, which inhibits tumor promoter-induced transformation of P+ cells, also enhances levels of TIS1 and TIS21 mRNAs in these cells.

The TIS21 gene has ~60% sequence similarity to another putative tumor suppressor, the BTG1 gene. The BTG1 gene is associated with a chromosomal translocation in a case of B-cell chronic lymphocytic leukemia, is expressed at high levels in density-arrested NIH3T3 cells, and exerts antiproliferative effects when overexpressed in NIH3T3 cells (Rouault et al., EMBO J 11:1663, 1992). We have compared the expression of BTG1 and TIS21 in TPA-treated JB6 cells. In contrast to the dramatic and preferential induction of TIS21 mRNA in P- cells, the BTG1 mRNA level is only moderately increased in response to TPA, and the level is similar in P+ and P- cells. The BTG1 and TIS21 genes, which may be members of a new family of tumor suppressor genes, clearly show different patterns of regulation of expression in response to TPA. In order to investigate whether these genes are differentially regulated throughout the cell cycle, their expression is being compared in density arrested and mitogenically stimulated JB6 cells.

Transfectant cell lines of P+ cells overexpressing TIS1 and/or TIS21 and of P- cells expressing antisense constructs of these genes are being generated to test the hypothesis that TIS1 and TIS21 inhibit the transformation process.

N 305 **THE AMINO ACID SEQUENCE OF THE MURINE DCC TUMOR SUPPRESSOR GENE.** Helen M. Cooper, Penny Armes, Joanne Britto, Ian Faragher, and Andrew F. Wilks. Ludwig Institute For Cancer Research, P.O.Box Royal Melbourne Hospital, Victoria, Australia 3050.

Colon cancer is one of the most prolific and malignant forms of human cancers. Recently, mutations have been identified in the DCC (Deleted in Colon Cancer) gene in over 70% of all colon carcinomas studied. Since it is mutated at both alleles in most carcinomas, the DCC gene appears to be a tumour suppressor gene. In general, these mutations are not observed until later stages of tumorigenesis suggesting that the loss of expression of the DCC gene product influences the metastatic and/or invasive phenotype of colorectal carcinoma. The DCC gene product shares strong homology at the amino acid level to members of the immunoglobulin supergene family. Many of these proteins are known to be homophilic cell adhesion molecules (eg. N-CAM, L1-CAM, Fasciclin I and III).

To date, only a partial sequence of the human DCC gene product has been reported (Fearon et al, 1989). The published sequence comprises 750 amino acids which encode 4 Ig-C2-like domains followed by 3 fibronectin type III repeats. Here we report the complete amino acid sequence of the murine DCC gene product derived from a mouse brain cDNA library. The murine DCC cDNA contains 4 Ig-C2 domains and 6 fibronectin type III repeats followed by a transmembrane and cytoplasmic domain. The mouse DCC amino acid sequence is 96% homologous to the reported human sequence. Since the DCC gene product is so highly conserved between mouse and man it is likely that DCC will play a central role in regulating aspects of cellular proliferation and/or differentiation.

Fearon, E., Cho,K.R., et al (1989) Science 247:49-56.

N 306 **REGULATION OF AN ANGIOGENESIS INHIBITOR IN HUMAN FIBROBLASTS BY THE P53 TUMOR SUPPRESSOR GENE.** Dameron, K.M., Volpert, O.V. and Bouck, N. Northwestern University Medical School,Chicago, IL 60611

The tumor suppressor gene p53 plays an important role in the progression of more than fifty percent of human tumors of various origins. While insight has been gained on possible biochemical functions of the p53 protein, the activities under its control that are important for progression of a tumor cell *in vivo* are still not clearly defined.

To determine if p53 plays a role in the switch to an angiogenic phenotype that is required for progressive growth of all solid tumors, fibroblasts from 3 different individuals with the Li-Fraumeni familial cancer syndrome were obtained. Early passage fibroblasts cultured from LiFraumeni patients are nontransformed, mortal and are heterozygous for p53 (p53 wt/m). When serially grown in culture, these fibroblasts spontaneously immortalize and express only mutant p53, analogous to what occurs in the tumors of these patients (Bischoff et. al., Oncogene (6) 183-186). The angiogenic phenotype of these cells was analyzed using an in vivo cornea assay and an in vitro endothelial cell migration assay. While conditioned media from early passage fibroblasts were non-angiogenic due to the presence of an inhibitor, late passage conditioned medias were angiogenic and had lost this inhibitory activity. The inhibitor was identified as thrombospondin (TSP), a secreted glycoprotein found in the extracellular matrix. Early passage mortal cells expressed high levels of TSP, while late passage immortal cells expressed 20-100 fold less TSP RNA and protein. To show TSP was responsible for the inhibitory activity, inactivating anti-thrombospondin antibodies were added to medias conditioned from early passage cells, this resulted in the loss of inhibitory activity. To directly demonstrate p53 regulation of TSP expression, late passage immortalized cells were transfected with the temperature-sensitive p53 plasmid. Only at the temperature where p53 is wild-type did the transfected cells express increased levels of TSP RNA and were non-angiogenic.

These experiments demonstrate a new function for the p53 tumor suppressor gene, regulation of angiogenesis. Since subpopulations of cells which gain the ability to be angiogenic should be at a selective advantage *in vivo*, these results offer one explanation for why the wild-type p53 gene is so often lost during progression of human tumors.

N 307 TRANSFECTION OF SV40T ANTIGEN AND TEMPERATURE-SENSITIVE p53 GENES INTO CULTURED HUMAN BUCCAL EPITHELIAL CELLS, R.C.Grafström[1], K.G.Wiman[2] and P.S.Kulkarni[1]. Institute of Environmental Medicine, Division of Toxicology[1] and Tumor Biology[2], Karolinska Institute, S-171 77 Stockholm, Sweden.

Normal human buccal epithelial cells can be grown on fibronectin/collagen-coated dishes and transferred in serum-free MCDB 153 medium containing defined mitogens and pituitary extract. The cells express epithelial features, divide at about 1 population doubling (PD) per day and commonly undergo 50 to 70 PD. Following transfer and expression of the SV40 large T antigen gene (SV40T), an apparently immortalized line termed SVpgC2a which has undergone more than 700 PD developed from one of several strains with extended life span. All SV40T-transfected strains exhibited strong nuclear expression of the SV40T and p53 proteins, whereas they differed in their morphology and expression of differentiated epithelial marker, i.e. keratin and involucrin. Karyotype analysis indicated variable increases in chromosome number in all strains except in SVpgC2a, which showed a hypodiploid pattern. Each of the strains responded in a different manner than normal cells to the growth modulating agents transforming growth factor-ß1, serum and elevated Ca^{2+}. In this regard, the development of a progressive resistence was evident from comparison of SVpgC2a with its parental substrain and normal cells. None of these strains were tumorigenic when injected subcutaneously in athymic nude mice, whereas a p53-negative buccal carcinoma line (SqCC/Y1) was found to induce tumors. Supertransfection of SVpgC2a with a mouse temperature-sensitive p53 gene construct (tsp53-Val135), gave rise to a substrain, which exhibited both normal and mutant specific p53 expression at 37°C for at least 4 months, indicating stable integration of this tsp53. Maintainance of these cells at 32°C, a temperature where this p53 mutant protein exhibits mainly wild type configuration, markedly decreased the growth rate as compared to the appropriate controls. A transient expression of the tsp53-val135 could be obtained in the SqCC/Y1 carcinoma line, since the expression was lost from most of the cells after 3 months. Finally, an attempt to transfect normal cells with tsp53-val135 did not result in phenotypically altered strains. Taken together, several SV40T-expressing buccal epithelial cell lines that show extended lifespans (including both mortal and immortal variants), chromosomal abnormalities and modulated responsiveness to factors that alter growth are now available and cryopreserved for further experimentation. Moreover, a continuous line is available in which expression of both wild type and mutant p53 can be regulated to a significant extent. The various strains now provide a battery of model systems that will be useful in studying mechanisms of human oral carcinogenesis, in particular the role of the commonly altered p53 gene.

N 308 MULTISTAGE CARCINOGENESIS *IN VIVO*: ASSESSMENT OF p53 LOSS IN TRANSGENIC MICE EXPRESSING V-FOS AND V-RAS^{Ha} EXCLUSIVELY IN THE EPIDERMIS. David A. Greenhalgh, Xiao-Jing Wang, Joshua N. Eckhardt and Dennis R. Roop. Departments of Cell Biology and Dermatology, Baylor College of Medicine, Houston, TX 77030

By means of an epidermal specific targeting vector based on human keratin 1 (HK1), transgenic mice were developed that expressed v-fos and/or v-ras^{Ha} exclusively in the epidermis. Transgenic mice expressing either HK1.ras or HK1.fos developed preneoplastic hyperplasia/hyperkeratosis, and later benign, regression prone papillomas. In mating experiments, HK1.fos/ras co-expressors exhibited a greater severity of pre-neoplastic phenotypes and immediate onset of tumorigenesis. However, while HK1.fos/ras co-expression was able to achieve an autonomous papilloma phenotype, malignant conversion was not observed, suggesting the requirement for an additional event(s). As loss of p53 is associated with malignant conversion in mouse skin, we mated HK1.ras and HK1.fos animals to null p53 mice to assess the effect of p53 loss on papilloma progression. Preliminary data show that HK1.ras or HK1.fos hemizygous p53 genotypes are phenotypically identical to wt p53 HK1 transgenic siblings. However, surprisingly, null p53 genotype siblings exhibit a delayed, reduced tumorigenesis, graphically demonstrated by HK1.ras/null p53 TPA promotion experiments. Thus in regression-prone papillomas, p53 loss is insufficient to achieve a detectable effect before null p53 genotypes succumb to spontaneous sarcomas. This result is consistent with a late role for p53 in mouse skin carcinogenesis. Thus the requirement for an autonomous papilloma phenotype prior to a role for p53 loss will be tested as HK1.fos/ras/null p53 genotypes are generated.

N 309 SUPPRESSION OF TUMORIGENICITY IN A HUMAN COLON CANCER CELL LINE BY INTRODUCTION OF A NORMAL *APC* GENE, Joanna Groden,[1] Nitai P. Bhattacharyya,[2] Geoff Joslyn,[3] Lisa Spirio,[2] Mark Meuth[2] and Ray White[2], [1]Department of Molecular Genetics, Biochemistry and Microbiology, The University of Cincinnati College of Medicine, Cincinnati, OH 45267; [2]The Eccles Institute of Human Genetics, The University of Utah, Salt Lake City, UT 84112; [3]The Fred Hutchinson Cancer Research Center, Seattle, WA 98104

The *APC* gene, mutations in which are responsible for the inherited colon cancer syndrome adenomatous polyposis coli (APC), is described as a tumor suppressor gene. This is based on the formal genetics of the disease and mutational analyses of sporadic colorectal tumors. A full-length, wild-type *APC* gene was introduced by transfection into human colon carcinoma cell lines. Clonal cell lines were derived and studied. Some of these cell lines exhibited an altered morphology as compared to the parental and control cell lines: they were larger, polygonal in shape and contained prevalent micronuclei. Many cells contained large vacuoles. One of these cell lines, HCT 116, has at least one normal copy of the *APC* gene, contains full-length APC protein and displays genomic instability at dinucleotide repeat loci. This cell line, following transfection with *APC*, yielded a large number of cell clones with an altered morphology. Some of these clones also had longer population doubling times than the parental cell line and were unable to grow colonies in soft agar. Lastly, injection of these cell clones into athymic mice resulted in no tumor formation, unlike the injection of parental cells or cells transfected with the vector and selectable marker alone. These results provide direct evidence that the *APC* gene can alter the transformation properties of colon carcinoma cells.

N 310 CELLULAR TRANSFORMATION BY G1 CYCLINS, Philip W. Hinds, Scott Eastman and Kathryn M. Latham, Department of Pathology, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115.

Cyclins activate cyclin-dependent kinases (cdks) to control transition through different phases of the cell cycle. The decision to progress from G1 phase to S phase involves the D and E type cyclins, which activate cdk4 and cdk2 and possibly others. Among the targets of these cyclin/kinase complexes is the retinoblastoma protein, pRb. pRb can prevent cell cycle progression in its hypophosphorylated state, perhaps by sequestering growth promoting proteins like the transcription factor E2F. Phosphorylation and inactivation of pRb can be driven by cyclin E/cdk2 complexes which are activated in late G1. D-type cyclins may also participate in this process, but are further implicated in pRb's function by their ability to form physical complexes with pRb. Indeed, overexpression of these D-type cyclins can inhibit pRb's suppressive function, and mutation of the pRb-binding domain of cyclin D1 does no alter this function. It is therefore possible that pRb negatively regulates cyclin D1 as it does E2F. Interestingly, cyclin D1 has been observed to be overexpressed in a number of tumor cells due to gene amplification and juxtaposition to cellular promoters. Indeed cyclin D1 was first identified as the PRAD-1 oncogene involved in parathyroid tumors, and may be identical to the bcl-1 oncogene in lymphoma. We have recently demonstrated that cyclin D1 can indeed act as an oncogene in primary rat cells by cooperating with a mutant adenovirus E1A protein defective in binding to pRb. This result supports a role for D-type cyclins in tumorigenesis that involves inactivation or circumvention of pRb. We have investigated the role of other cyclins in this assay, and show that cyclins D2, D3 and E can each complement the mutant E1A protein, but cyclins A and B1 cannot. Further, analysis of pRb in the transformed cells shows no change of its phosphorylation status in cells expressing high levels of D-type cyclins. In contrast, cyclin E seems to result in excessive hyperphosphorylation of pRb. We are currently investigating the function status of these pRb proteins in terms of E2F association and nuclear anchoring to determine if pRb is indeed the target of these cyclins in transformation. In addition, we have introduced E1A proteins defective in multiple CR2 functions into BRK cells with D-type cyclins and into pRb-minus primary murine fibroblasts to assay the relative roles of pRb, p107 and p130 in this transformation pathway.

N 311 MOLECULAR INDUCTION OF RETINAL TUMORS OR RETINAL DEGENERATION IN TRANSGENIC MICE. Kimberly A. Howes,[1] David S. Papermaster,[2] Nancy Ransom,[2] Jacques G.H. Lasudry,[3] Daniel M. Albert,[3] and Jolene J. Windle[4] [1]Dept. of Cellular & Structural Biology, University of Texas Health Science Center, San Antonio, TX, [2]Dept. of Pathology, University of Texas Health Science Center, San Antonio, TX, [3]Dept. of Ophthalmology, University of Wisconsin, Madison, WI, [4]Cancer Therapy & Research Center, San Antonio, TX

Retinoblastoma, a childhood malignancy of the retina, has been widely studied as a model for the predisposition to cancer. To facilitate studies on the molecular basis of retinoblastoma, we have developed several transgenic mouse lines in which the promoter to the interstitial retinol binding protein (IRBP) directs the expression of SV40 T-antigen or human papilloma virus (HPV) 16 E7 and E6 viral oncoproteins which differentially bind and inactivate the tumor suppressor proteins pRb and p53. IRBP efficiently directs the expression of these viral oncoproteins to retinal photoreceptor cells prior to their terminal differentiation. IRBP-Tag transgenic mice develop retinoblastoma of photoreceptor layer origin. However, the IRBP-E7 mouse line, which inactivates pRb while leaving p53 activity intact, exhibits retinal degeneration. Instead of differentiating, the photoreceptor cells of IRBP-E7 mice exhibit morphological features of apoptosis and exhibit DNA laddering, resulting in the loss of the photoreceptor layer. These results suggest that pRb may be essential for the differentiation of photoreceptor cells during retinal development. An additional transgenic mouse line, IRBP-E6, which inactivates p53 but not pRb, shows no obvious developmental retinal abnormalties. However, preliminary data from crosses between IRBP-E7 and IRBP-E6 lines reveal ocular tumors develop in offspring which contain both E7 and E6 transgenes. These trangenic mouse lines represent a unique model to examine the molecular mechanisms involved in the cellular decision to undergo tumorigenesis vs. cell death.

N 312 GENETIC BASIS OF PREDISPOSITION TO TUMOUR PROGRESSION IN MICE, Chris Kemp and Allan Balmain, The Beatson Institute for Cancer Research, Glasgow, Scotland, G61-1BD.

Extensive animal studies have shown that the process of carcinogenesis can be divided into the stages of initiation, promotion and progression. We have utilized *p53*knockout mice to learn more about the role of this gene in two of the best characterised multistep carcinogenesis models: the murine skin and liver. Groups of wild type, *p53*heterozygous and *p53*null mice were treated on the dorsal skin with DMBA followed by twice weekly treatments with TPA. The number, size and growth rate of papilomas was identical in the wild type and heterozygous mice. However, the progression rate to carcinomas was increased three fold in the heterozygotes and this was associated with the loss of the remaining wild type allele. There were fewer papillomas in the null mice but these progressed very rapidly to carcinomas. The great majority of carcinomas were markedly more malignant and undifferentiated compared with wild type tumours. Thus inactivation of *p53*does not increase initiation or promotion but is a major rate limiting step for tumour progression. To determine what role *p53*might play in hepatocarcinogenesis we treated groups of wild type and *p53*heterozygous male mice at 12 days of age with a single injection of the liver carcinogen DEN. The genetic background of the mice as well as endogenous levels of testosterone provide a strong promoting stimulus. The number, size and growth rate of preneoplastic lesions, measured from 18 to 26 weeks of age, was not different between the groups. Also, up to 32 weeks of age there was no difference in the number of visible liver tumours between the groups. These tumours were largely benign in appearance and unlike the squamous cell carcinomas in the heterozygous mice, none had lost the remaining wild type *p53*allele. In conclusion, a reduction of *p53*gene dosage does not provide a growth advantage to either benign skin or liver tumours, but complete loss of *p53* is rate limiting for malignant progression.

N 313 THE NF2 TUMOR SUPPRESSOR GENE: MUTATIONS IN MULTIPLE TUMOR TYPES AND INITIAL CHARACTERIZATION OF ITS ENCODED PRODUCT Nikolai Kley,Albert Bianchi,Tetsuo Hara,Andre Klein-Szanto^,Vijaya Ramesh*,James F. Gusella*, R. Lekanne Deprez# Ellen Zwarthoff#, and Bernd R. Seizinger. Dept. Molecular Genetics and Cell Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton,NJ 08543, ^Fox Chase Cancer Center,Philadelphia,Pennsylvania 19111, * Neurogenetics Laboratory,Mass. Gen. Hospital, Charlestown,MA 02129,#Department of Pathology, Erasmus University,Rotterdam DR 3000 The Netherlands.

Neurofibromatosis type II is an inherited disease characterized predominantly by the occurrence of bilateral vestibular schwannomas. Other tumor types typically associated with NF2 include meningiomas, spinal neurofibromas, and certain astrocytic tumors in the brain. These tumor types also occur as sporadic tumors in the general population and comprise the vast majority of human nervous system neoplasms. Here we describe a novel NF2 gene transcript isoform encoding of protein with altered C terminus, and show that a high frequency of somatic mutations in NF2 gene transcripts are associated with sporadic vestibular schwannomas and meningiomas. The results are consistent with the notion that the NF2 gene acts as a recessive tumor suppressor gene. Furthermore, mutations were detected in breast carcinoma (ductal/lobular) and melanoma, tumor types not typically associated with NF2 but known to be associated with chromosome 22 deletions. Mutations comprise point mutations, deletions (in frame, and frame shift), and insertions. In the most part ,these mutations predict to encode truncated versions of the NF2 gene product (merlin), with deletions at both the N or C terminus. Structure-function relationships will be discussed. In summary, it appears that mutations in the NF2 gene are associated also with seemingly NF2-unrelated sporadic cancers in the general population, suggesting that it may play a more widespread role in the development of human cancers other than brain tumors.Results on the initial characterization of the merlin protein product (protein purification and analysis of cellular merlin), will be presented.

N 314 HUMAN RETINOBLASTOMA GENE PRODUCT PREVENTS c-Ha-*ras* ONCOGENE MEDIATED CELLULAR TRANSFORMATION OF MOUSE FIBROBLASTS, Marikki Laiho, Laura Kivinen, and Kimmo Pitkänen, Department of Virology, University of Helsinki, FIN-00014 Helsinki, Finland

Suppression of tumor formation and restoration of normal growth of cells has been an insignia that the retinoblastoma gene (*RB1*) functions as a tumor suppressor gene. Since tumorigenesis may involve dysfunction of both oncogenes and tumor suppressor genes we have addressed the question whether the presence of RB protein can suppress transformation elicited by oncogenes. For this purpose we have generated stable mouse fibroblast clones expressing either full-length human wild-type (wt) or mutant pRB. In contrast to certain tumor cells, the expression of wt or mutant RB protein did not affect the growth properties of fibroblasts under normal culture conditions. These cell lines were used to analyse the interactions of RB and c-Ha-*ras* oncogene by gene transfection studies. Mouse fibroblasts stably expressing high levels human wt pRB or mutant pRB were transfected with genomic or LTR promoter driven c-Ha-ras(Val-12) expression vectors. We find that expression of wt, but not mutant RB protein in the cells prevents c-Ha-*ras* oncogene mediated cellular transformation and colony formation in soft agar. Analysis of stable RB and genomic c-Ha-*ras* cell transfectants for expression of pRB and $p21^{ras}$ by immunoblotting indicates a strong correlation with the presence of high levels of RB protein and inhibition of ras-transformation. Moreover, during culturing the RB and genomic c-Ha-*ras* expressing clones a progressive transformation of phenotypically normal clones was observed which paralleled loss or decrease of RB expression and concomitant increase in $p21^{ras}$ production. These findings suggest a functional cross-talk between RB protein and $p21^{ras}$, which balances the cell phenotype between normal and transformed states.

N 315 PROTEIN EFFECTS OF MUTANT P53 EXPRESSION IN A MURINE P53 NULL CELL LINE. Laura M. Madrid, Gerard T. Zambetti and Donald A. Young. Departments of Environmental Medicine and Medicine, University of Rochester, Rochester NY 14642 Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38101

The p53 gene is a tumour suppressor, the mutation of which is implicated in carcinogenesis. Mutant p53 proteins differ from wild-type p53 and from each other in their localization, conformation and transforming potential. To identify mutant p53 responsive proteins we have analyzed a murine 10(3) cell line which lacks endogenous p53 and five derivative cell lines. These each have a different mutant p53 protein stablely introduced into them and contain no normal p53. Whole cell lysates and supernatants for all cell lines have been analyzed by giant 2-D gel electrophoresis and protein changes identified . It was observed that these mutations share protein changes common to each other. Groups of proteins were observed to either be induced or suppressed depending upon the individual characteristics of the mutation such as transforming ability and location of mutation on the p53 protein. Because these cells are not expressing a wild type p53 protein these changes may be predominantly due to mutant p53 function. To further investigate and confirm these mutant p53 protein changes we are in the process of transfecting wild type p53 into the null and mutant cell lines. This will further help to identify protein changes due to mutant p53 function.

Supported by NIEHS grant ES07026 and NIH grant CA-56833

N 316 THE TUMOR SUPPRESSORS NF1 AND NF2: THEIR ANTI-RAS ACTIONS AND FUNCTIONAL DOMAINS.

Hiroshi Maruta[1], Mikael Varga[1], Vijaya Ramesh[2] and James Gusella[2], [1]Ludwig Institute for Cancer Research, Melbourne, and [2]Massachusetts General Hospital, Boston.

NF1 and NF2 are the neurofibromatosis type 1 and type 2 gene products of 2818 and 595 amino acids, respectively. Dysfunction or deletion of NF1 or NF2 causes development of schwannomas and other tumors in human brain.

NF1 is a Ras GTPase activating protein (GAP), and contains a domain of 327 amino acids (residues 1205 to 1531) called NF1-GRD which shares 26% sequence identity with the C-terminal domain (GAP1C, residues 720 to 1044) of another Ras GAP called GAP1. Both NF1-GRD and GAP1C activate normal Ras GTPases, but not oncogenic mutants such as V-HaRas. We have previously shown that NF1-GRD as well as its short fragment of 91 amino acids (NF91, residues 1441 to 1531) reverse V-HaRas-induced malignant transformation of NIH/3T3 cells (Nur-E-Kamal et al; J. Biol. Chem. 268 (1993), in press). NF1-GRD is the first Ras-binding protein, and so far NF91 is the smallest, among the tumor suppressor proteins that have shown the anti-V-HaRas action in mammalian cells, respectively. In this paper we demonstrate that its smaller fragment of only 56 amino acids (NF56, residues 1441 to 1496) is still able to reverse the V-HaRas-induced malignant transformation. Since the NF56 cannot activate either normal Ras GTPase or its oncogenic mutants, its anti-V-HaRas action is not due to the GTP to GDP conversion of V-HaRas but due to its tight binding to V-HaRas and thereby preventing V-HaRas from its interacting with a downstream target(s).

NF2 is related to an F-actin capping protein called Radixin at their N-terminal halves. Radixin binds the fast growing end of actin filament (F-actin) and induces the shortening of the filament. Since another F-actin capping protein called gelsolin can reverse V-HaRas-induced malignancy, we are currently examining the possible anti-Ras action of NF2 and its small fragments.

N 317 DEVELOPMENT OF CANCER CACHEXIA SYNDROME AND ADRENAL TUMORS IN INHIBIN-DEFICIENT MICE, M.M. Matzuk, M.J. Finegold, J.P. Mather, L. Krummen, H. Lu, and A. Bradley. Dept. of Pathology and Inst. for Molecular Genetics, Baylor College of Medicine, Houston, TX 77030, and Dept. of Cell Culture Research and Development, Genentech, Inc., South San Francisco, CA 94080

The inhibins are α:β heterodimeric growth factors which are members of a large family of growth regulatory proteins that includes the activins, Mullerian inhibiting substance and the TGF-β's. These proteins appear to play important roles in multiple tissues as endocrine, paracrine and autocrine mediators. Using a gene targeting/embryonic stem cell approach, we have previously demonstrated that inhibin can function as a tumor suppressor in the gonads of mice. In this study, we show that the development of the gonadal tumors is rapidly followed by a severe wasting syndrome which mimics the cancer cachexia syndrome accompanying many human cancers. Mice which are gonadectomized at an early age do not develop this wasting syndrome confirming that a factor secreted by the tumors is directly or indirectly causing these effects. In addition, prolongation of the life of these mice by the gonadectomy reveals that these inhibin-deficient mice ultimately develop adrenal cortical tumors. These studies suggest that inhibin-deficient mice are an important mammalian model for understanding cancer cachexia syndrome and identify inhibin as a novel tumor suppressor in the adrenal gland.

N 318 INTRODUCTION OF HUMAN CHROMOSOME 11 SUPPRESSES THE TUMORIGENICITY OF ADENO TRANSFORMED BABY RAT KIDNEY CELLS; INVOLVEMENT OF THE WT1 GENE? A.L.Menke, E.Sonneveld, R.C.A. van Ham, A.G.Jochemsen, E.J.Stanbridge*, F.J.Rausscher III#, A.J. van der Eb. Medical Biochemistry, Sylvius laboratory, wassenaarseweg 72, 2333 AL Leiden, The Netherlands. (*University of California, Irvine) (#The Wistar Institute, Philadelphia)

The adenovirus-transformation system has proven to be very usefull in the study of carcinogenesis, differentiation and the regulation of gene expression. Using this system, important information has been obtained about essential cell cycle genes like Rb, cyclins and cdk's. We have started to investigate whether the adeno-transformation system could also be used in the study of tumor suppressor genes. With microcell mediated chromosome transfer, we introduced several human chromosomes into tumorigenic Adeno transformed baby rat kidney cells (BRK's). It was found that, upon introduction of human chromosome 11 or the short arm of chromosome 11 (11p), the tumorigenicity of the adeno-transformed BRK's is reduced when injected into nude mice. Experiments with use of the recently described deletion chromosomes (Dowdy et al.1991, Science . 254, 293) suggest that region 11p13 is involved in the reduced tumorigenicity. A candidate suppressor gene is the Wilms' tumor 1 (WT1) gene which is located in this region. This is supported by Southern analysis and PCR analysis which show loss of the WT1 gene expression in the tumors which eventually arise after injections of the microcell hybrids. The WT1 gene is one of at least three genes involved in the origin of Wilms' tumor , an embryonic malignancy of the kidney. Evidence sofar indicate that the WT1 protein exerts its activity as a transcriptional regulator. It has been shown that it binds to specific DNA sequences and in this way could repress the transcription of several growth related genes. In order to prove that WT1 is involved in suppression of tumorigenicity, we transfected WT1 cDNA constructs into the adeno-transformed BRK's. WT1 cDNA can be expressed in Adeno-transformed BRK cells after transfection. The effect on tumorigenicity, upon introduction of the cDNA constructs, will be presented.

N 319 ASSOCIATION OF HUMAN AND MOUSE CYTOPLASMIC p53 WITH HEAT SHOCK PROTEINS, B. Alex Merrick, Chaoying He, Lora L. Witcher, Rachel M. Patterson and James K. Selkirk, Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

P53-associated proteins (p53-AP) are being actively investigated for possible relationships to p53 function. Several cellular proteins bind to p53 including MDM2, HSP70, replication protein A, TATA binding protein, CCAAT binding factor and others. While, discernment must be made between binding proteins which act as sequestering agents or as functional partners, consideration should also be also given to associated proteins which relate to p53 translational processing and cellular translocation. For these reasons, immunoprecipitation (IP) studies with PAb421 were performed in ^{35}S-labeled nuclear and cytoplasmic lysates from C3H10T1/2 murine and HT1080 human lines to study potential subcellular differences in p53-AP's. Nuclear p53 was generally free from other proteins while p53 in cytoplasm was associated with proteins of 55 kD, 70 kD and 90 kD mass. Separation of p53-AP by 2D PAGE using IP and western blotting provided identification for cytoplasmic p53-AP's as HSC70, HSP70, GRP78 and HSP90. When anti-HSP70 or anti-HSP90 MAb were used in IP, each co-precipitated the other but not p53, suggesting an HSP70:HSP90 complex exclusive of other proteins. Due to the complexity of the HSP70 pattern, we further investigated the nature of p53-AP HSP70's by protein sequencing. Sequencing from preparative 2D PAGE blots confirmed the identity of GRP78 as well as a new HSP70 class protein, also called GRP75 or Mot-1. Other HSP70's were N-terminal blocked. In the nucleus, IP and western blots suggested HSP70 and HSP90 proteins were free and did not exist as a complex. IP of p53 from nuclear lysates showed the association of only a small amount of HSP90 and no other p53-AP. We believe these cytoplasmic p53-AP's reflect constituitive p53 processing and that the p53:HSP70:HSP90 complex may be chaperonin in nature. Components of p53 cytoplasmic complex parallel those elements proposed for the steroid-receptor transportosome, suggesting a role of cytoplasmic p53-AP in cellular translocation.

N 320 TARGETED MUTAGENESIS OF APC GENE IN MICE.
S. Miura[1], J. Kuno[1], K. Jishage[1], Y. Nakamura[2] AND T. Noda[1].
[1]Department of Cell Biology and [2]Department of Biochemistry, Cancer Institute, 1-37-1 Kami-ikebukuro, Toshima-ku, Tokyo, Japan.
Adenomatous poliposis coli (APC) gene was cloned as a gene responsible for the development of numerous number of polyps of familial adenomatous poliposis coli (FAP) patients. Various types of germ-line mutations have been found in the APC gene of FAP patients. Lots of sporadic colorectal cancers have also been shown to carry somatic mutations in APC gene. These data suggest that APC gene is a tumor suppressor gene. To obtain a direct answer if such mutations of APC gene lead to the development of colorectal tumors, we have generated mouse lines which harbor a mutated APC gene by the targeted mutagenesis in ES cells. First, we cloned the mouse APC gene using human APC gene cDNA and analyzed the structure of mouse APC gene. Then, we constructed a targeting vector in a way that we could obtain mutant APC gene product truncated at codon 1309 as a result of homologous recombination. After electroporation of ES cells with the vector, cells were cultured with the medium containing G418 and subcultured. We picked up 650 colonies and screened their genomic DNA with Southern blotting. Eighteen ES clones were shown to undergo homologous recombination and five of them were injected into mouse blastocysts to generate chimeras. We obtained germ-line chimeras from all ES clones injected and those chimeras, together with the heterozygous offsprings, were analysed whether they would suffer from tumors. In five chimeras, we observed bleeding from lower intestinal tract. Such chimeras were sacrificed and tumors in intestinal tract were observed. We will report the result of analysis of these tumors generated in large intestine of chimeric mice.

N 321 TARGETED MUTATION OF THE *mdm2* GENE IN EMBRYO-DERIVED STEM CELLS,
Roberto Montes de Oca Luna and Guillermina Lozano, Department of Molecular Genetics, M. D. Anderson Cancer Center, Houston, Tx 77030.
The *mdm2* gene behaves as an oncogene in its ability to cooperate with activated *ras* to induce transformation. The *mdm2* protein can bind to the p53 protein and inhibit both its transactivation function and its growth-suppressive properties. In addition, the oncogene *mdm2* has been shown to be amplified in several types of sarcomas containing wild-type p53 protein. Although *mdm2* can be a negative regulator of p53, its real function is still unknown. To understand the function of *mdm2*, we sought to generate *mdm2*-deficient mice by knocking out the gene via homologous recombination in embryonic stem (ES) cells. We first isolated the *mdm2*-cDNA by PCR from a thymus cDNA library and used it as a probe to screen a mouse 129/SvE genomic library. Two overlapping λ clones were isolated and analyzed to identify the exon-intron boundaries of the *mdm2* gene. Based on the structure of the *mdm2* genomic gene, we designed a targeting vector that deletes almost one half of the gene including its zinc finger domain. Targeted ES cell clones have been identified and will be used to generate chimeric mice. *mdm2*-deficient mice will help to understand the function of *mdm2* gene.

N 322 CONNEXIN 32 GENE MUTATION IN A RAT LIVER TUMOR AND ITS INVOLVEMENT IN CARCINOGENESIS,
Yasufumi Omori, Nikolai Mironov, Vladimir Krutovskikh, Hiroshi Yamasaki, Unit of Multistage Carcinogenesis, International Agency for Research on Cancer, Lyon France,
Gap junctional intercellular communication (GJIC) is one of the main mechanisms in maintaining tissue homeostasis, and its abortion can lead to aberrant cell growth. A number of studies have suggested that the reduced GJIC is involved in carcinogenesis. However, little is known about the cause of this reduction.
We examined the gene alteration of connexin 32 (cx32), which is expressed chiefly in epithelial cells, in 13 chemically-induced rat liver tumor samples. PCR and single strand conformation polymorphism (SSCP) were applied to detect mutations. 8 overlapped fragments covered the whole coding region of cx32 gene.
One tumor showed an abnormal pattern on SSCP. DNA sequencing of the abnormal band revealed one base mutation (G to A) at codon 220, resulting in one amino residue change (Arg to His). This mutation was located in C-terminal of cx32 (cytoplasmic region). This is the first example of a mutated connexin gene in tumor samples. Immunohistochemical study on this sample showed that cx32 proteins were localized not in cell membrane but in nucleus.
According to low frequency of mutation, it is unlikely that mutation is a general reason for the abortion of GJIC in tumor tissues. However, these results suggest that the mutation in C-terminal cytoplasmic region may cause the abnormal intranuclear localization, in other words, this region is essential for precise sorting of cx32 protein. This cytoplasmic part presents low homology among connexin families and seems to be a regulatory domain. Hereafter it will become important to investigate how this region contributes to formation and function of gap junction.

N 323 THE ROLE OF *p53* MUTATIONS IN MURINE MAMMARY PRENEOPLASIA,
Michelle A. Ozbun, Frances S. Kittrell*, Daniel Medina*, and Janet S. Butel. Division of Molecular Virology and Department of Cell Biology*, Baylor College of Medicine, Houston, Texas 77030.
Mutations in *p53* are common in a number of tumor types, including mammary cancers. We have employed a murine system to investigate the role that *p53* mutations may have in the early stages of breast cancer. Cell lines established from virgin BALB/c mice are capable of forming normal ductal outgrowths or hyperplastic alveolar nodules (HAN) when transplanted into cleared mammary fat pats of syngeneic animals. Both ductal outgrowths and HAN can be serially passaged *in vivo* and established as cell lines *in vitro*. The HAN represent a clear preneoplastic lesion in murine mammary tumorigenesis. We previously determined that HAN express mutant *p53* genes, and these mutations are maintained in tumors which arise from the HAN *in vivo*. However, the normal ductal outgrowths appear not to express mutant *p53*. To further establish a cause and effect relationship between *p53* mutations and preneoplasia *in vivo*, we have used retroviral vectors containing a selectable marker (*neo*) to attempt to alter the *p53* status of mammary cells. Primary mammary epithelial cells, cells from normal ductal outgrowths (EL11), and cells from HAN outgrowths (TM2H, which express no endogenous p53) were infected with retroviral vectors containing human wild-type *p53*, mutant R273H, or mutant R175H. The primary and EL11 cells were transplanted *in vivo* without G418 selection, whereas the TM2H cells were selected *in vitro* prior to transplantation. Following selection, a portion of the TM2H cells were analyzed for *p53* expression and growth characteristics *in vitro*. Our preliminary data indicate that the TM2H cells infected with R273H *p53* expressed high levels of p53; however, no p53 expression was detected in either the uninfected or the wild-type *p53*-infected TM2H cells. This correlates with finding no difference in growth rate or saturation density between the uninfected or wild-type p53-infected TM2H cells. Furthermore, expression of the *p53* mutant R273H did not provide the TM2H cells with any added growth advantage in this assay. The phenotypic changes associated with expression of wild-type or mutant *p53* in mammary outgrowths *in vivo* will be discussed.

N 324 TUMOR-SUPPRESSIVE ACTIVITY OF N03 GENE IN v-*src*-TRANSFORMED RAT 3Y1 FIBROBLASTS, Shigeru Sakiyama, Toshinori Ozaki and Hideki Enomoto, Division of Biochemistry, Chiba Cancer Center Research Institute, Chiba 260, J APAN.

A differential screening procedure was used to isolate genes whose expression was significantly down-regulated in transformed cells. After screening a cDNA library derived from normal rat fibroblast (3Y1) cells, we have obtained several independent cDNAs whose mRNA level was significantly lower in 3Y1 cells transformed with Rous sarcoma virus than in untransformed 3Y1 cells. Among them, N03 cDNA has been characterized extensively. The expression of N03 gene was also decreased in several types of transformed cells, including v-*mos*- or SV40-transformed 3Y1 cells. Nucleotide sequence analysis showed that N03 protein was composed of 178 amino acid residues and did not have any sequence similarity with proteins in the data base. A putative zinc-finger motif was located in the central part of the sequence and a proline-rich domain was in the COOH-terminal region. Genomic Southern experiment revealed that N03 gene exists as a single copy in the rat genome and that closely related, single copy genes are also present in chicken and human. To understand the possible role(s) of N03 gene, we have obtained transfectants expressing N03 gene from v-*src*-transformed 3Y1 cells. Two clones, which overexpressed N03 mRNA and maintained v-*src* mRNA as well as v-*src*-encoded tyrosine kinase activity, showed a flatter phenotype, a reduced rate in growth and a decreased ability to grow in soft agar. One of the clones which retained N03 expression was completely suppressed in forming tumors in nude mice. These data suggest that N03 gene may have a suppressive ability on transformed phenotypes. In addition, the nature of human N03 cDNA will also be discussed.

N 325 EFFECT OF THE Rb ON THROMBOSPONDIN TRANSCRIPTION IN NORMAL AND NICKEL-TRANSFORMED CELLS, Konstantin Salnikow and Max Costa, Department of Environmental Medicine and Kaplan Comprehensive Cancer Center, NYU Medical Center. New York, NY 10016

Nickel-transformed Chinese hamster embryo cells exhibit inactivation of a senescence gene present on the X chromosome. This activity was previously found to be regulated by methylation. We have used a new approach to subtract mRNA and isolate genes abundantly transcribed in normal cells but not in nickel-transformed cells with the hope of identifying senescence genes. One gene that was expressed in normal cells and less abundantly in nickel-transformed cells was thrombospondin. Thrombospondin gene expression was stimulated in normal cells by transfection of a plasmid expressing the Rb gene but this effect was less in nickel-transformed cells. These findings were obtained by using human thrombospondin promoter linked to a CAT reporter gene. Additionally, in nickel-resistant cells where the thrombospondin was transcribed at a much lower level than wild type cells, there was hypermethylation of the promoter region. Thus, the effect on the thrombospondin transcription in nickel-transformed Chinese hamster cells was probably a nickel-specific effect. Interaction of the Rb gene and its effect on transcription of thrombospondin is interesting with regard to its possible relationship with a senescence gene, since Rb interacts with other protein transcription factors.

N 326 cDNA LIBRARIES ENCODING GENES EXPRESSED DURING NORMAL NEURAL CREST DEVELOPMENT: REAGENTS FOR CLONING NOVEL TUMOR SUPPRESSOR GENES IN MELANOMA AND NEUROBLASTOMA. Melissa C. Southey*, Susan G. Bevan+, Jane E. Armes#, Joe Minichiello+, Donald F. Newgreen+ and Deon J. Venter*, *Department of Anatomical Pathology and +The Murdoch Institute for Research into Birth Defects, Royal Children's Hospital, Parkville, Vic, 3052; and #Department of Anatomical Pathology, Austin Hospital, Heidelberg, 3084, Victoria, Australia.

Tumors derived from the embryonic neural crest tissues (including neuroblastoma and melanoma) display several characteristic cytogenetic deletions consistent with dysfunction of tumor suppressor genes. Prolonged efforts to clone the genes affected by these deletions (using standard positional cloning strategies) have been only partially successful. We have therefore begun a novel strategy aimed at elucidating the nature of these putative tumor suppressor genes. The strategy is based on i) the evidence indicating that a proportion of these tumours arise as a result of disordered embryogenesis and / or abnormal precursor cell division and differentiation. and ii) the striking similarities at the molecular and cellular levels in neural crest development between vertebrates. Thus, following micro-dissection of neural crest tissue in quail embryos, we have generated cDNA libraries representing the genes expressed at the various stages of avian neural crest development, encompassing pre-migratory, migratory, and post-migratory (such as melanocyte) populations. These libraries are currently being used as: i) A source of probes to identify human chromosome - specific clones for in-situ hybridisation to areas of the human genome commonly deleted in these tumours, a strategy which is designed to provide candidate tumour suppressor genes which will be analysed further for evidence of abnormalities in tumour DNA; ii) Templates for PCR using degenerate primers to various classes of genes, including receptor tyrosine kinases, tyrosine phosphatases and cell adhesion molecules, resulting in the production of products which have been subcloned into M13 and are currently undergoing further sequence analysis and iii) Developmental biology studies. The results of the in - situ hybridisation and PCR - based experiments will be presented.

N 327 A REVERTANT OF mos-TRANSFORMED RAT CELLS EXHIBITS REDUCED LEVEL OF MAP KINASE ACTIVITY AND IS RESTRICTED TO RETRANSFORMATION BY SERINE/THREONINE KINASE ONCOGENES. Lilia Z. Topol[1], Maria Marx[2], Georges Calothy[2], and Donald G. Blair[1], [1]Laboratory of Molecular Oncology, National Cancer Institute, Frederick, MD, USA; [2]Institut Curie-Biologie, Centre Universitaire, 91405 Orsay Cedex, France

Despite significant progress in elucidating v-mos function, little is known about the mechanism by which v-mos induces cell transformation. In order to study this mechanism, we isolated and characterized a flat (non-transformed) revertant from cells transformed by Moloney mouse sarcoma virus (Mo-MuSV) expressing the v-mos oncogene. We generated doubly mos-transformed rat fibroblasts (DTM) by initially transforming immortalized rat fibroblasts with the ts Mo-MuSV and then with wild type Mo-MuSV at the non-permissive (non-transforming) temperature for the ts virus. We subsequently isolated a transformation suppressed revertant cell line (F-1), following transfection of DTM cells with a retroviral construct (pIC4Neo) containing the neo gene under the control of the RAV-1 LTR, and selection of G418-resistant colonies. Like the DTM parental cell line, the F-1 revertant contained two integrated copies of v-mos, expressed elevated levels of mos-specific RNA, and contained rescuable transforming viruses. The F-1 revertant did not grow in soft agar and showed a greatly reduced ability to form tumors in nude mice. Revertant cells were resistant to retransformation by v-mos, by v-raf, and by v-src, but could be efficiently retransformed by ras (Ha-MSV). Recent studies have shown that v-mos can interact with the cellular proteins, tubulin and mitogen-activated protein (MAP) kinase. Revertant cells exhibited organized tubulin and actin structures similar to that found in normal cells. In contrast, MAP kinase ERK2 activity, which is elevated in v-mos-expressing cells, including DTM, is reduced to below normal, non-transformed cell levels in the F-1 revertant. This suggests that the kinase cascade responsible for the mos-dependent activation of MAP kinase is altered for this transformation-resistant revertant. Further analyses of mos function in revertant cells are in progress, and possible mechanisms of mos suppression in F-1 cells, will be presented.

N 328 P53-DEFICIENCY AND v-ras^{Ha} COOPERATE IN MALIGNANT CONVERSION OF KERATINOCYTES. WC Weinberg, N Kadiwar, CG Azzoli, DL Morgan, S Littles and SH Yuspa, National Cancer Institute, Bethesda, MD 20892.

The p53 gene is frequently mutated in chemically- and UV-induced epidermal carcinomas. We have studied epidermal keratinocytes from mice expressing a null mutation in the p53 gene (Donehower et al, Nature 356:215,1992) to determine the role of p53 in epidermal growth regulation and carcinogenesis. The p53 status of newborn mice was determined by PCR analysis of tail DNA. Keratinocyte cultures from p53-deficient newborns and wildtype littermates were infected with a replication-defective retrovirus encoding the v-ras^{Ha} oncogene and grafted onto nude mice; p53(-/-) cells developed into carcinomas (8/8), while p53(+/-) and p53(+/+) cells formed papillomas. The average size of p53 (+/+), (+/-) and (-/-) tumors 3 weeks following grafting was 575±155 (n=12), 1710±303 (n=21) and 1643± 647 (n=7) mm^3, respectively. By 5 weeks, 10/19 p53(+/-) and 0/8 p53(+/+) tumors converted to carcinomas. Control and v-ras^{Ha} cultures of each genotype were also studied *in vitro*. Uninfected cells of each genotype changed morphology, expressed differentiation-specific markers, and decreased ligand binding to EGF receptors when induced to differentiate by 1.2mM Ca^{2+} and ^{3}H-thymidine incorporation was decreased by 94-96% after 24 hours. However, following introduction of v-ras^{Ha}, p53(+/+), (+/-) and (-/-) cells cultured in 1.2mM Ca^{2+} incorporated 3.0%, 7.6%, and 17.4% ^{3}H-thymidine, respectively, of infected cultures maintained in 0.05mM Ca^{2+}. p53(-/-) cells expressing v-ras^{Ha} were also less responsive to the growth-inhibitory effects of TGF-beta. These results suggest that altered growth regulation of p53-deficient cells expressing the v-ras^{Ha} oncogene may be due in part to decreased responsiveness to negative growth regulators.

N 329 DETECTION OF RETINOBLASTOMA GENE DELETIONS IN RADIATION-INDUCED MOUSE LUNG ADENOCARCINOMAS, Gayle E. Woloschak, Mark E. Churchill, Yueru Zhang and M. Anne Gemmell, Center for Mechanistic Biology and Biotechnology, Argonne National Laboratory, 9700 South Cass Ave., Argonne, IL 60439

From 1971 to 1986, Argonne National Laboratory conducted a series of large-scale studies of tumor incidence in 40,000 BCF_1 mice irradiated with ^{60}Co γ rays or JANUS fission-spectrum neutrons; normal and tumor tissues from these mice were preserved in paraffin blocks. A polymerase chain reaction (PCR) technique was developed to detect deletions in the mouse retinoblastoma (*mRb*) gene in the paraffin-embedded tissues. Six *mRb* gene exon fragments were amplified in a 40-cycle, 3-temperature PCR protocol. The absence of any of these fragments (relative to control PCR products) on a Southern blot indicated a deletion of that portion of the *mRb* gene. The tumors chosen for analysis were lung adenocarcinomas that were judged to be the cause of death in post-mortem analyses. Spontaneous tumors as well as those from irradiated mice (569 cGy of ^{60}Co γ rays or 60 cGy of JANUS neutrons, doses that have been found to have approximately equal biological effectiveness in the BCF_1 mouse) were analyzed for *mRb* deletions. In all normal mouse tissues studied (18 total), all six *mRb* exon fragments were present on Southern blots. Tumors in six neutron-irradiated mice also had no *mRb* deletions. However, 1 of 6 tumors from γ-irradiated mice (17%) and 6 of 18 spontaneous tumors from unirradiated mice (33%) had a deletion in one or both *mRb* alleles. All deletions detected were in the 5′ region of the *mRb* gene. More recent experiments examining neutron dose-rate effects have demonstrated differences in the frequencies of *mRb* lesions in mice exposed to the same dose of neutrons administered at low vs. high dose rates. Work supported by the U.S. Department of Energy, Office of Health and Environmental Research, under Contract No. W-31-109-ENG-38.

N 330 SV40 T ANTIGEN (tsA58) CAUSES THROMBOCYTOPENIA IN TRANSGENIC MICE. Wen Zhou, Martha Hokom, Dimitry Danilenko, Pamela Hunt, Robert Bosselman and Murray O. Robinson. Amgen, Inc., Thousand Oaks, California 91320.

Platelets function in the circulation to aid in the process of blood clotting. Patients undergoing chemotherapy often lose appropriate levels of circulating platelets (thrombocytopenia) resulting treatment complications. To better understand the formation of platelets and the maintenance of circulating platelet levels we sought to perturb this process *in vivo*. We generated transgenic mice expressing the tsA58 mutant of the SV40 T antigen via the megakaryocyte-specific Platelet Factor 4 promoter. More than half of the seventeen transgenic lines established displayed chronic thrombocytopenia. The degree of thrombocytopenia varied among lines, yet within each line the platelet levels remained stable. The platelet deficiency appeared to correlate with transgene expression, as homozygotes exhibited a more severe platelet reduction than hemizygotes within any given line. Western blots also suggest that T antigen protein levels correlate with phenotype. Affected lines have megakaryocyte morphology similar to those observed in experimentally induced models of thrombocytopenia, displaying increased size and ploidy. Additionally, megakaryocyte numbers were inversely correlated with the platelet level.

The role of Retinoblastoma protein (Rb) in the observed phenotype was investigated by mating the transgenic mice to mice heterozygous for the Rb knockout. In at least some lines, transgenic mice with an inactivated copy of the Rb gene had even lower platelet counts than those with both copies of the Rb gene.

In older mice (>7months) megakaryocytic neoplasia, characterized by striking splenomegaly and neoplastic infiltration into many tissues, was often observed in lines with both normal and reduced platelet counts. Infrequently, other tumors of unknown cell type have been seen, suggesting the possibility of transgene expression in other tissues.

These findings indicate that T antigen is interfering with terminal differentiation of the megakaryocyte lineage, and that T antigen target proteins such as the Retinoblastoma (Rb) gene product may be important for normal differentiation of megakaryocytes. Investigations into the role of Rb and p53 in megakaryocyte maturation and attempts to establish megakaryocyte cell lines are currently underway.

N 331 Identification of cooperating events in the generation of lymphoid tumors in *(lpr,lpr)* and normal transgenic mice

M. Zörnig, T. Schmidt, A. Grzeschiczek and T. Möröy, Institut für Molekularbiologie und Tumorforschung, Philipps Universität Marburg, Emil-Mannkopff-Str. 2, D-35037 Marburg, Germany

Lymphoid specific coexpression of the PIM-1 serine threonine kinase and one member of the MYC family of transcription factors as transgenes in mice leads to the rapid development of malignant B- or T-cell tumors. While the combination c-MYC/PIM-1 has the most drastic effect provoking outbreak of the disease already at neotatal stages, L-MYC/PIM-1 double transgenics develop tumors after an average latency period of about 12 weeks. All tumors emerging from these double transgenics develop clonally as judged from the presence of distinct rearrangements of the T-cell receptor genes, clearly indicating the requirement of additional stochastic events. To identify such events that cooperate with the activated *L-myc* and *pim-1* transgenes, we performed a proviral tagging experiment with MoMuLV -a non acute transforming retrovirus. Infection of neonatal *L-myc/pim-1* double transgenic mice provokes now the development of T-cell tumors with a shortened latency period of 6-7 weeks. Analysis of these tumors revealed the activation of the loci *pal-1*, *evi-5*, *gfi-1* and of several uncharacterized loci through proviral integration. To date, the nature of *pal* and *evi* genes remains to be determined, but the *gfi-1* gene is known to encode a Zink-finger containing transcription factor that was shown to render T-cells independant from IL-2. Similar to the mice tagged with MoMuLV, *lpr,lpr* mutant mice carry an endogenous retroviral Etn element that has integrated into the gene for the FAS receptor and blocks its expression. The FAS receptor is able to transduce apoptotic signals in T-cells, and its absence in *lpr,lpr* mice leads to a nonmalignant proliferation of abnormal T-cells. Expression of an *L-myc* transgene in these mice can accelerate tumorigenesis, suggesting that the loss of FAS and the expression of MYC can cooperate in tumor formation. In contrast, expression of a *pim-1* transgene in these mice did not accelerate tumorigenesis but dramatically enhanced the lymphoproliferation typical for *lpr,lpr* mice by further inhibiting apoptosis in T-cells, suggesting that PIM-1 can at least under these circumstances interfere with apoptosis. Therefore, we hypothesize that the triple cooperation of MYC as a stimulator of cell proliferation, GFI-1 that confers growth factor independance and PIM-1 that inhibits apoptosis, is most effective in the generation of lymphoid malignancies.

Genomic Instability and Clinical Implications I

N 400 AN ANALYSIS OF LOSS OF HETEROZYGOSITY AT 5q21 IN LUNG CANCER, Vivien J Bubb, Nicola Smithson, Sarah Gledhill, Frank Carey, Andrew Wyllie and Colin C Bird. CRC Laboratories, Department of Pathology, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, Scotland

The tumour suppressor gene, adenomatous polyposis coli (APC) located at 5q21 has been identified as the gene linked to inheritance of familial polyposis coli. It is also inactivated in over 60% of sporadic colorectal carcinomas. Recent studies from various laboratories have demonstrated loss of heterozygosity of this locus, (which also contains a second putative tumour suppressor gene, mutated in colon cancer (MCC)), in a number of other types of primary tumour including renal, oesophageal, hepatic and lung carcinomas.

This study demonstrates a clear correlation between loss of heterozygosity at this locus and histological type of lung cancer. Gene loss is common in squamous and small cell carcinomas but rare in adenocarcinomas. As in colorectal cancer, loss of either MCC or APC alone is a rare event. Current studies on the mutation status of APC will determine whether this gene is specifically inactivated in squamous carcinoma of the lung.

N 401 ALTERATION IN THE PHOSPHORYLATION OF THE Rb PROTEIN IN NICKEL- TRANSFORMED HUMAN CELLS, Max Costa, W. Karol Dowjat and Xinhua Lin, Department of Environmental Medicine and Kaplan Comprehensive Cancer Center, NYU Medical Center, New York, NY 10016

Retinoblastoma protein has been studied in various crystalline NiS transformed cell lines that were derived from the human cell line HOS TE-85. The parent cell line was not able to grow in soft agar while following treatment with crystalline NiS compounds, cells were altered such that they were able to proliferate in soft agar media. The retinoblastoma protein appeared only in the unphosphorylated form in 8 out of 9 nickel-transformed clones examined while in the parental cells the Rb appeared in both phosphorylated and unphosphorylated forms. Rb protein levels and its phosphorylation were not affected by nickel treatmentor in nickel-resistant cells. Nickel-transformed clones expressed major regulators of Rb phosphorylation, cyclin E and cdk-2 at similar levels to parental cells. In co-immunoprecipitation assays, the transformed clones tested exhibiting the hypophosphorylated form, failed to form a complex with SV40 T large antigen indicating a lack of functional activity. When a plasmid containing the normal Rb gene was transfected into nickel-transformed cells, it restored the Rb phosphorylation pattern observed in parental cells, suggesting that a mutation was induced in nickel-transformed cells affected the ability of the protein to be phosphorylated and function normally.

N 402 GENETIC ANALYSIS OF TUMOR PROGRESSION IN SV40 T ANTIGEN TRANSGENIC MICE, William A. Held, Jodi A. Giancola O'Brien, Kimberly A. Kerns, Joanne Pazik, Kenneth W. Gross, and Curt Sigmund*, Department of Molecular and Cellular Biology, Roswell Park Cancer Institute, Buffalo, NY 14263; and *Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, IA 52242

To identify genetic loci potentially involved in tumor progression, tumor DNAs from SV40 T antigen transgenic, interspecific F1 hybrid mice were analyzed for loss of heterozygosity (LOH). In transgenic mice containing a liver-specific major urinary protein enhancer/promoter regulating expression of SV40 T antigen (MT-D2), liver tumor formation is preceded by dysplastic liver growth, apoptosis, and multifocal hyperplasia. Renin/SV40 T antigen mice (TAG4) develop sudden, sporadic, rapidly growing subcutaneous and occasional perirenal tumors. Tumor DNAs were analyzed for LOH at 24 loci on 17 chromosomes by Southern blotting. The liver tumors exhibited a broad spectrum of LOH with frequent losses of loci on ch 1, 5, 7, 8, and 12. LOH of the distal region of ch 7 involve preferential loss of the maternally inherited allele. This region is known to be imprinted and may correspond to human 11p15.5 associated with Wilm's tumor and Beckwith-Wiedeman syndrome which also involves preferential loss of the maternal allele. On the other hand, LOH on chromosome 8 involves preferential loss of the paternally inherited allele. In contrast, analysis of subcutaneous tumors in renin/SV40 T antigen transgenic mice indicated a very restricted pattern of LOH confined almost entirely to loci on ch 8. The very frequent ch 8 LOH (16/21 tumors) also exhibited a pronounced preference for loss of the paternal allele (14/16). Approximately one-half of these tumors also have amplifications of more proximal ch 8 alleles, including Junb and/or Jund. Our results indicate that genetic instability is a prominent feature of tumorigenesis in SV40 T antigen transgenic mice with LOH and gene amplification potentially playing important roles in tumor progression. The allele-specific losses suggests involvement of parentally imprinted genes and/or allele-specific differences in tumor susceptibility genes.

N 403 HOMOZYGOUS HA-RAS MUTATIONS OCCUR IN 4NQO INDUCED MURINE ORAL SQUAMOUS CARCINOGENESIS, Fred J. Hendler, Bo Yuan, Briana W. Heniford, and Douglas M. Ackermann, Departments of Medicine, Biochemistry, Surgery, and Pathology, the James Graham Brown Cancer Center, University of Louisville, Louisville VA Medical Center, and Alliant Hospitals, Louisville, KY 40292

To determine if Ha-*ras* mutations occurred during malignant transformation, CBA mouse oropharyngeal mucosa were treated with the chemical carcinogen, 4-nitroquinoline-1-oxide (4NQO) from 4 to 16 weeks. Tissues were obtained from mice after ≥24 weeks of observation. The first and second exons of Ha-ras genes were amplified from cryosections by PCR and subsequently reamplified by nested PCR. Using a mismatch amplification mutation assay (MAMA), single strand conformational polymorphism (SSCP) and restriction fragment length polymorphism (RFLP) analysis, the mutant Ha-*ras* DNA was detected in 13 of 25 tissue specimens (10/14 invasive carcinomas, 2/4 carcinoma in situ; 1/5 dysplastic tissue; 0/2 normal tissues). DNA sequencing demonstrated the mutation was a G to A transition at codon 12. No codon 11,13 or 61 mutations were observed. The mutant allele was homozygous in 5 of the 15 invasive cancers. Homozygous Ha-*ras* mutations were observed in larger tumors. These results are consistent with a causal relationship between the carcinogen treatment, Ha-*ras* activation, and the initiation of tumorigenesis. The presence of homozygous Ha-ras lesions in the larger invasive squamous cell carcinomas suggests that gross chromosomal mutations occur in mouse chromosome 7 after neoplastic transformation has occurred and in the absence of carcinogens.

N 404 THE EFFECT OF ADENOVIRUS E1A AND E1B ONCOPROTEINS ON GENOMIC STABILITY, Suzanne M. Hess and Thea D. Tlsty, Department of Pathology and UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC 27599

Normal cells possess pathways that maintain genomic integrity. These pathways may involve cell cycle checkpoints. If these pathways become disrupted, either spontaneously, virally, or environmentally, genomic instability may result. One chromosomal alteration that is a manifestation of genomic instability is gene amplification. The amplification potential of a cell may be determined by measuring the ability of a cell to become resistant to the drug PALA, an inhibitor in the pyrimidine biosynthetic pathway. The major mechanism of resistance to PALA is through amplification of the CAD gene, a trifunctional enzyme involved in pyrimidine biosynthesis. Normal diploid cells lack a detectable frequency of CAD gene amplification. In contrast, preneoplastic, nontumorigenic cells have a detectable amplification frequency, while tumorigenic cells have a high amplification frequency. Therefore, it appears acquisition of genomic instability may be a primary step in tumorigenesis. If the loss of a cell's ability to maintain its genomic integrity is a primary factor in developing cancer, then understanding the regulation of genomic instability is extremely important in the study and prevention of cancer.

Both p53 dependent and p53 independent pathways have been identified in the abrogation of checkpoint control. This work focuses on these pathways by manipulating normal cell regulation with viral oncoproteins. Studies with the E6/E7 oncoproteins of human papillomavirus (HPV), and the SV40 large T antigen (SV40 T Ag), have shown that these viral oncoproteins disrupt genomic integrity. Studies with the E1A and E1B oncoproteins introduced into normal human fibroblasts (NHF) will be presented. These oncoprotein expressing NHF cells have been assayed for genomic instability. The E1A oncoprotein interacts with the cellular proteins pRb, p107, p58 cyclin A, p130, p300, and p33 cdk2, while E1B interacts with p53. By using viral oncoproteins to identify components involved in maintaining genomic stability, this research will provide insight into the events that disrupt this integrity during the neoplastic process.

N 405 EXPRESSION OF ENDOGENOUS WILD-TYPE p53 IN G_1 AND G_2M IN CULTURED LUNG EPITHELIAL CELLS FOLLOWING EXPOSURE TO ALPHA PARTICLES AND X RAYS, Neil F. Johnson, Albert W. Hickman, and John F. Lechner, Inhalation Toxicology Research Institute, Albuquerque, NM 87185

X rays have been shown to increase the expression of wild-type p53, which is associated with cell-cycle arrest in G_1. We have examined the effect of ^{238}Pu alpha particles and of X rays on the expression of p53 in cultured lung epithelial cells irradiated with graded doses of alpha radiation (0.0, 0.01, 0.05, 0.1, 0.5, 1.0, and 2.0 Gy) and X rays (0.0, 0.05, 0.1, 0.5, 1.0, and 2.0 Gy). The fraction of cells expressing increased amounts of p53 was determined by indirect immunohistochemistry using a monoclonal antibody (clone 241) combined with flow cytometry. Cells were also stained with propidium iodide for cell-cycle analysis. Irradiation by alpha particles and X rays induced a dose-dependent increase in p53 expression. p53 was maximally expressed 4-6 h after both types of irradiation. Analysis of X-ray-exposed cells showed that the majority of cells expressing increased amounts of p53 were located in G_1 (21% at 1.0 Gy) with fewer cells in G_2M (7% at 1.0 Gy). Alpha-radiation-exposed cells expressed increased amounts of p53 in G_1 (41% at 1.0 Gy) and in G_2M (15% at 1.0 Gy). This difference may be related to a greater propensity of alpha irradiation to cause chromosomal damage. [Research supported by US DOE/OHER under Contract No. DE-AC04-76EV01013]

N 406 CHROMOSOME 8P LOSS OF HETEROZYGOSITY DEFINED BY MICROSATELLITE REPEAT MARKERS: FREQUENT DELETIONS IN COLORECTAL AND LUNG CARCINOMAS. Kelemen, P.R., Yaremko, M.L., Kim, A., Minna, J. and Westbrook, C.A. Department of Medicine, University of Chicago, Chicago, IL and Simmons Cancer Center, UT Southwestern Medical Center, Dallas, TX.

Deletions from chromosome 8p have been seen in many tumors, including liver, lung, colorectal and prostate, suggesting the presence of a tumor suppressor gene (TSG) at this location. To construct a deletion map of the region involved, we typed 7 colorectal cancers microdissected from paraffin blocks and 11 lung carcinoma cell lines with a panel of 10 microsatellite repeat polymorphisms for 8p. The markers were: D8S259, D8S131, D8S137, NEFL, SFTP2, D8S133, LPL5', D8S261, D8S252 and D8S201. Deletions were frequent, seen in all colorectal carcinomas and 6 of 10 non-small cell lung carcinomas, but were notably absent from small cell lung carcinomas. The deletions seen in lung and colon cancer were localized to approximately the same regions of 8p21-22, with a proximal boundary at D8S131 and a distal boundary of SFTP2. We conclude that a region of common deletion exists between SFTP2 and D8S131, and that a TSG involved in these two tumors localizes here.

N 407 THE ABOLITION OF RADIATION INDUCED G1 GROWTH ARREST IN A HUMAN FIBROBLAST CELL LINE TRANSFECTED WITH THE HPV E6 GENE. Chuan Li, Hatsumi Nagasawa, Ngan-Ming Tsang, and John B. Little, Laboratory of Radiobiology, Harvard School of Public Health, Boston, MA02115

We have shown previously that some fibroblast cell strains from families predisposed to retinoblastoma exhibit an unusually large radiation induced G1 phase block when compared with normal diploid fibroblasts (Little *et al.* Cancer Res. 49, 4705-4714, 1989). In light of recent findings that the tumor suppresser gene p53 may play an important role in the G1/S delay observed in response to DNA damaging agents, we have examined the effects of the human papilloma virus E6 protein on the G1 arrest response in a heterozygous Rb cell strain. This strain, derived from skin fibroblasts of a 18 month old boy diagnosed with hereditary retinoblastoma at 8 months of age, showed a large G1 arrest response after radiation exposure. By use of the cumulative labeling index method with [^{3}H]thymidine, nearly 60% of the cells were found to be arrested in G1 after exposure to 400 cGy of gamma radiation. A plasmid p1436 containing the gene for the E6 protein of the human papilloma virus 16 was transfected into these cells for stable incorporation into the host genome. Nearly 100% of cells from a transfected strain expressing E6 progressed into S phase with no delay. These results suggest that the p53 mediated pathway to radiation response was abolished by the introduction of the HPV E6 protein. This is in agreement with other worker's results that the E6 protein can bind and mediate the degradation of the p53 protein. The molecular status of the p53 protein in the wild-type and transfected cell strains is currently under investigation.

N 408 p53 PHYSICALLY ASSOCIATES WITH CDK2 IN A CELL CYCLE-DEPENDENT MANNER, Chou-Chi H. Li,[1] Ren-Ming Dai[1] and Dan L. Longo,[2] Biological Carcinogenesis and Development Program, Program Resources, Inc./Dyncorp,[1] Biological Response Modifiers Program, Division of Cancer Treatment,[2] NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702

Alteration of p53 tumor suppressor gene is the most widely detected mutation in human cancers. The deregulation of cell cycle progression is closely associated with p53 mutations. p53 protein functions as a negetive regulator of cell proliferation and its activity is involved in the G1/S transition. Its activity appears to be modulated by phosphorylation.

In this study, we show that p53 is physically associated with and probably regulated by p33-Cdk2 kinase in a cell cycle-dependent manner. Specifically at G1/S transition, we also detected 1) activation of Cdk2 enzyme, 2) p53-associated kinase activity, 3) phosphorylation of p53, 4) nuclear localization of the active form of Cdk2, and 5) nuclear localization of the phosphorylated p53. These observations suggest that Cdk2 protein may be the physiological kinase that phosphorylates p53.

N 409 A CELL CYCLE CHECKPOINT FUNCTION IN NORMAL, IMMORTAL AND TRANSFORMED RODENT HEPATOCYTES WITH THE WILD TYPE P53 GENE, Katsuhiro Ogawa, Kunihiko Tsuji and Takayuki Kadohama, Department of Pathology, Asahikawa Medical College, Asahikawa, Japan

Recent evidence suggests that p53 participates in a cell cycle checkpoint in DNA-damaged cells that may provide a sufficient time for cells to repair DNA damage. We investigated a cell cycle checkpoint function after DNA damage in normal, immortal and transformed rodent hepatocytes, all of which contained the wild type p53 gene. When primary-isolated neonatal rat hepatocytes were exposed to a nonlethal dose of UV, actinomycin D or mitomycin C, most cells expressed abundant p53 with an abnormally-extended half life in their nuclei, and their growth was arrested despite treatment with growth factors (EGF plus insulin). When DNA damaged cells were treated with p53 antisense oligonucleotides, the p53 expression was significantly suppressed, and an appreciable fraction of the cells entered S phase. When damaged cells were treated with p53-sense or Rb-antisense oligonucleotides, such recovery from growth arrest was not observed. The data suggest that p53 is indeed a component of signal transduction pathway(s) leading to growth arrest in normal hepatocytes after DNA damage.

On the other hand, when primary-isolated hepatocytes of C3H mice were cultured for a long period, liver epithelial cell clones which can indefinitely grow in vitro were developed. When the activated H-ras gene was introduced into those cells, transformed clones which were highly tumorigenic could be isolated. Both immortal and ras-transformed cells contained the wild type p53 gene. When these cells were exposed to the DNA damaging agents, both immortal and ras-transformed cells expressed abundant p53 with extended half life as observed in normal rat hepatocytes. However, growth arrest was distinct only in immortal cells, but less prominent in the ras-transformed cells. The results suggest that, although p53 dependent cell cycle checkpoint function is maintained in immortal cells, it was inactivated in the ras-transformed cells in spite of the presence of wild type p53 gene.

N 410 EFFECTS ON THE IN VITRO AND IN VIVO GROWTH PROPERTIES OF A HUMAN BREAST CARCINOMA CELL LINE AND A WILMS' TUMOR CELL LINE FOLLOWING MICROCELL-MEDIATED TRANSFER OF A NORMAL HUMAN CHROMOSOME 16, Karen K. Phillips, Julia T. Arnold, and Bernard E. Weissman, Department of Pathology, Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599

Cytogenetic and molecular studies have shown that losses of both chromosome 11p and 16q alleles are nonrandom events in Wilms' tumor and breast carcinoma tumor samples, suggesting that these chromosomal regions carry tumor suppressor genes. Since inactivation of a tumor suppressor gene on chromosome 16 may be an important step in the initiation and progression of these two cancer types, we are determining if the introduction of a normal 16 into representative cell lines affects tumorigenicity and metastasis in vivo and the transformed phenotype in vitro.

We have introduced a normal human chromosome 16 into the mouse A9 cell line for use in microcell hybrid studies with the MDA-MB-435 breast carcinoma cell line and G401.6TG Wilms' tumor cell line. The microcell hybrids will be analyzed for the in vitro changes of population doubling time and growth in soft agar and for changes in tumorigenicity by subcutaneous injections in nude mice. Because loss of heterozygosity in 16 and 11 are associated with several other human tumors, including rhabdomyosarcoma, hepatoblastoma, non-small-cell lung carcinoma, and ovarian carcinoma, studies on the biological effect of tumor suppressor genes involved in breast carcinoma and Wilms' tumor could have important implications for tumorigenic mechanisms in general.

N 411 **PARALLEL CHANGES IN p53 AND PCNA RESPONSES TO X-IRRADIATION IN HUMAN BLADDER CANCER CELL LINES, Julio C. Ribeiro, Janelle Hanley, Pamela J.Russell, Oncology Research Centre, Prince of Wales Hospital, NSW, 2031, Australia.**

The recently established human bladder cancer cell lines, UCRU BL 13 (BL 13), UCRU BL 17 (BL 17) and UCRU BL 28 (BL 28) were analysed for p53 mutations and expression. Each line gave similar results by immunoprecipitation and Northern analysis. However, SSCP analysis covering exons 4-10, the region of the p53 gene most commonly mutated in cancer cells, revealed a base change in intron 6 of BL-17, which was confirmed by sequencing to be 62 bases after the border of exon 6 (G to A). No mutations were detected by SSCP analysis in exons 4-10 in BL 13 or BL 28. These lines, together with a control line, CALU 6 (no p53), derived from a lung carcinoma, were subjected to X-irradiation over a dose range of 0-20 Gy to determine the effects of DNA damage on p53 expression, assessed by flow cytometric analysis of p53 protein 4 hours later. As expected, CALU-6 cells failed to express p53. The BL 17 cells showed an increase in p53 levels which fluctuated over a 2 fold range in median fluorescence intensity after exposure to different doses of X-irradiation whereas the BL-13 and BL-28 bladder cancer cells showed minimal changes in the levels of p53 after X-irradiation. We are currently investigating the X-ray sensitivity of these lines. The proliferation of the cells was monitored by analysis of PCNA expression also by flow cytometry. In each bladder cancer cell line, changes in p53 levels with irradiation dose were mirrored by parallel changes in PCNA levels. Although wt p53 has been shown to cause growth arrest in G1, and inhibits the promotion of the PCNA gene, mutant p53 has been shown to increase PCNA promoter activity (Deb, S. *et al*, J. Virology, **66**:6164, 1992). Our results suggest that expression of an apparently normal p53 gene in BL 13 and BL 28 cells or of a p53 gene with a mutation in intron 6 in BL 17 cells, can also increase the expression of PCNA. Reasons for this, such as mutations outside exons 4-10, or interactions of p53 with other important proteins such as MDM2 and GADD45 in these cells, are currently under investigation. Alternatively, PCNA may have a direct role, along with p53, in repair of DNA damaged by X-irradiation. This is being tested by studying X-irradiation of synchronized cells.

N 412 DELETION MAPPING OF CHROMOSOME 19 IN ASTROCYTOMA, OLIGODENDROGLIOMA, AND MIXED OLIGOASTROCYTOMA.

Ritland SR†, Ganju V‡, Jenkins RB†. † Mayo Clinic, Dept. of Laboratory Genetics, Rochester MN 55905, ‡ Geelong Hospital, Dept. of Oncology, Victoria Australia 3220.

Allelic mutation on chromosome 19 has been reported by several groups as a common genetic event in human glial tumors. In an effort to localize specific regions of importance on this chromosome, a total of 26 polymorphic genetic markers were used to evaluate loss of heterozygosity (LOH) and microsatellite instability in 75 astrocytomas (57 grade 4, 5 grade 3, 5 grade 2, 6 grade 1, and 2 other), 18 oligodendrogliomas (5 grade 4, 12 grade 2, and 1 grade 1), and 8 mixed oligoastrocytomas (MOA) (3 grade 4, 2 grade 3, 3 grade 2). No microsatellite expansion was observed in these tumors for any of the chromosome 19 loci examined. LOH on chromosome 19 was detected in 23/75 astrocytomas (31%), 11/18 oligodendrogliomas (61%), and 5/8 MOA (63%). Partial deletions of chromosome 19 were observed for 19 astrocytomas, 10 oligodendrogliomas, and 4 MOA. In 14/14 instances of partial deletion for oligodendrogliomas and MOA, the 19q arm was found to have LOH while the 19p arm showed no loss. In 14/19 instances of partial deletion for astrocytomas, the 19p arm showed LOH while the 19q arm showed no loss. Thus loss of 19q and retention of 19p is associated with oligodendroglioma and MOA, while loss of 19p and retention of 19q is associated with astrocytoma ($p < 0.01$). These data, in combination with the high rates of LOH observed on chromosome 19 for these tumors, indicate that two or more tumor suppressor genes may reside on chromosome 19; one on 19p that may be important for the development of astrocytomas and one on 19q that may be important in oligodendrogliomas and MOA.

N 413 ANALYSIS OF MUTATION FREQUENCY IN p53-DEFICIENT MICE,

Arthur T. Sands*, Milind B. Suraokar*, Lawrence A. Donehower+ and Allan Bradley*, *Department of Molecular Genetics, +Department of Virology, Baylor College of Medicine, Houston, TX 77030

Mice deficient in p53 made by homologous recombination have been mated to the transgenic mice carrying a lambda shuttle vector containing a lacI gene as a target in order to investigate the effect of p53 -/- genotype on mutation frequency. These transgenic mice (Big Blue, Stratagene) have previously been used to for *in vivo* measurement of mutations in multiple tissues and to test the mutagenic effects of several compounds. It has been previously shown that approximately 74% of mice homozygous for the p53 mutation have developed tumors in a wide range of tissues by six months of age (Donehower, et al., Nature, vol. 356, 1992). In addition, p53 deficiency has been associated with a decreased apoptotic and growth arrest response after exposure to DNA damaging agents. It has been hypothesized, therefore, that lack of p53 contributes to cell survival with mutation after DNA damage. To test this hypothesis, analysis of mutation frequency was performed on DNA from thymus and spleen from p53(-/-)/Big Blue Mice. In addition, embryonic fibroblasts were derived from embryos that contain a p53 -/- mutation and the lambda shuttle vector. These fibroblasts were analyzed for colony forming ability and mutation frequency after exposure to DNA damaging agents. Preliminary experiments indicated that these cells exhibited marked growth rate differences and altered cell cycle control by flow cytometry. In addition, early studies of cells homozygous for the p53 deficiency have demonstrated a decreased sensitivity to DNA damaging agents in a colony survival assay as compared to wild-type controls. Flow cytometric studies have also revealed different cell cycle responses to DNA damaging agents between wild-type and p53-/- cells. Wild-type cells contained an increased fraction of hypofluorescent cells which might be associated with apoptosis while p53-/- cells exhibited an increase in aneuploidy.

N 414 CHARACTERIZATION OF THE VON HIPPEL-LINDAU TUMOR SUPPRESSOR PROTEIN, Thomas M. Stackhouse*, Steven Giardina*, Jami Lipan#, Mary Lou Orcutt#, Kalman Tory*, Fuh-Mei Duh*, Michael Lerman#, Berton Zbar#. *Program Resources Inc./DynCorp and #Laboratory of Immunobiology [F.L., M. Y., I. K., M. L. O., F. Z., J. L., M. I. L., B. Z.], National Cancer Institute- Frederick Cancer Research and Development Center, Frederick, MD. 21702

Von Hippel-Lindau disease (VHL) is a multi-system neoplastic disorder characterized by a predisposition to develop tumors in the brain, spinal cord, eyes, kidneys and adrenal glands. Using a positional cloning strategy, we have isolated a partial cDNA that is the VHL tumor suppressor gene (Science 260: 1317-1320; 1993).

This gene shows high evolutionary conservation and encodes two variant transcripts of 6 and 6.5 Kb. The predicted protein sequence contains 8 copies of a tandemly repeated pentamer (GXEEX) that shows 48% homology to a repeat found in the procyclic surface membrane glycoprotein of Trypanosoma brucei.

A partial protein sequence was expressed in E. coli. The amino acid sequence of the partial VHL protein was confirmed by amino terminal sequencing and used to produce both rabbit polyclonal antibodies and mouse monoclonal antibodies. Using these antibodies to examine the protein expression in tumor and normal tissue as well as different cell lines, we observed low levels of expression in most cell types. However, on several samples the level of expression was high, showing a doublet with a size of 55/60 Kd.

N 415 MECHANISMS OF GENOMIC INSTABILITY IN FIBROBLASTS WITH GERMLINE P53 MUTATIONS, Michael A. Tainsky, Philip Liu, Tian-Ai Wu, and Louise C. Strong, Department of Tumor Biology, University of Texas, M.D. Anderson Cancer Center, Houston, Texas 77030

We have characterized normal cells from inherited cancer patients with Li-Fraumeni syndrome for the purposes of understanding mechanisms of carcinogenesis and to identify the inherited gene. We have observed the selection of certain cells that spontaneously transform into infinite lifespan cell lines in culture under conditions in which cells from normal, unaffected controls were clearly limited in their growth and number of cell divisions. Fibroblasts from 7 out of 7 affected individuals from five Li-Fraumeni families develop changes in morphology, chromosomal abnormalities, and enter a growth crisis during which they begin to senesce (in a fashion similar to fibroblasts from normal donors) but then they recover. Chromosomal abnormalities occur even prior to their emergence from senescence; essentially all metaphases examined contained abnormal chromosome numbers, numerous damaged chromosomes and evidence for gene amplification in the form of double minute chromosomes and homogeneously staining regions. This suggests that the cells are prone to spontaneous mutations that predispose them to undergo further changes leading to their immortalization. Based on our in vitro studies of normal dermal fibroblasts from the most striking probands from candidate families we postulated that p53 might be the inherited gene responsible for Li-Fraumeni syndrome. Germline p53 mutations were found in five of five families. The immortal fibroblasts lose the wild type p53 upon escape from senescence and restoration of a wild type gene results in senescence and cell death. Cell cycle analyses indicate that p53 regulates a check point in G1 which can be restored in transfectants expressing low levels of wild type p53. In appropriate entry into S may give rise to genomic instability. Transfection of mutant p53 genes into normal diploid fibroblasts from a normal donor gives rise to genomic instability including double minute chromosomes, aneuploidy and extended lifespan as observed in Li-Fraumeni syndrome fibroblasts. Shuttle vector studies on the effect of p53 on mutation rates and fidelity of replication reveal that expression of a mutant and wild type p53 is more mutagenic that expression of a mutant p53 alone.

N 416 GENETIC MAPPING OF VARIOUS PROTEIN TYROSINE PHOSPHATASES ON HUMAN AND MOUSE CHROMOSOMES, A. van den Maagdenberg*, J. Rijss*, D. Olde Weghuis#, J. Schepens*, A. Verhofstad&, A. Geurts van Kessel#, B. Wieringa* and W. Hendriks*. Departments of *Cell Biology & Histology, #Human Genetics, and &Pathology, University of Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

Protein tyrosine phosphatases (PTPases) are a family of recently discovered enzymes, which catalyze dephoshorylation of phosphotyrosine residues. Being the antagonistic counterparts of the well known protein tyrosine kinases, PTPases are potential tumorsupressors. Hence, loss of activity may be involved in neoplastic transformations. We plan to investigate the role of PTPases in tumor initiation and progression by determining both their chromosomal localisation and tissue-type expression pattern. From these data, combined with knowledge on chromosomal aberrations in human tumors, predictions will be made on possible PTPase gene loss in certain tumor types. Subsequently, genetic markers will be generated for the PTPase locus involved, so that loss of heterozygosity can be tested in the appropriate tumors. For several PTPase family members, including LAR and PTPδ and a new, brain specific, PTPase, the localisation on both human and mouse chromosomes and the generation and use of markers will be presented.

N 417 GENOMIC INTEGRITY,CELL CYCLE CONTROL, AND CELL DEATH IN NORMAL HUMAN FIBROBLASTS EXPRESSING HPV ONCOPROTEINS, Alicia E. White*†, Elizabeth Livanos*, and Thea D. Tlsty*†, *Lineberger Comprehensive Cancer Center, Department of Pathology, and †Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC 27599-7295

Genomic integrity is maintained by a network of cellular activities that assesses the status of the genome at a given point in time and provides signals to proceed with or halt cell cycle progression. Mutations in any part of these cellular pathways can have the ultimate effect of disrupting chromosomal integrity. In this study we use viral proteins involved in malignant transformation to investigate cellular pathways that may be perturbed during loss of genomic stability. Recent studies have indentified cellular proteins which are targets for the viral oncoproteins, stressing the importance of these cellular proteins in controlling neoplasia. Among the targets of the viral oncoproteins are the products of the p53 and retinoblastoma (Rb) tumor suppressor genes. We demonstrate that the expression of human papillomavirus type16 E6 and E7 oncoproteins in normal, mortal cells disrupts the integration of the network of signals that maintain genomic integrity. E6-expressing cells, in which cellular p53 protein is bound and degraded, exhibited alterations in cell cycle control and displayed the ability to amplify the endogenous CAD gene when placed in the drug PALA. Expression of E7, which complexes with a variety of cellular proteins including Rb, resulted in an p53-independent alteration in cell cycle control, massive cell death, and polyploidy upon PALA treatment. These results demonstrate that the viral proteins disrupt cellular processes that safeguard the genome and growth of normal cells.

Genomic Instability and Clinical Implications II

N 500 P53 MUTATIONS ASSOCIATED WITH ANAPLASTIC WILMS' TUMOR, A HISTOLOGICAL SUBTYPE WITH POOR PROGNOSIS, Nabeel Bardeesy[1], David Falkoff[1], Mary-Jane Petruzzi[2], Bernhard Zabel[3], Norma Nowak[2], Tom Shows[2] and Jerry Pelletier[1], [1]Dept of Biochemistry, McGill University, Montreal, PQ, Canada H3G 1Y6, [2]Roswell Park Memorial Institute, State University of New York at Buffalo, New York, [3]Dept of Pediatrics, University of Mainz, Mainz, Germany.

The genetics of Wilms' Tumor (WT), a pediatric malignancy of the kidney, is complex and likely to involve several loci. Mutations in the WT1 tumor suppressor gene, located at chromosome 11p13, are associated with the development of a small proportion of WTs. Linkage data suggests the existence of other WT loci at chromosome 11p15 and at regions distinct from chromosome 11p. Chromosome 17p alterations have been detected in cytogenetic studies of WTs. We have scanned WTs for mutations in the p53 gene, located at 17p13, and found to be the most frequently mutated gene in human cancers. We analyzed the p53 gene in 140 WTs. Seven of the tumors were found to have p53 mutations. There was a very strong correlation between the presence of anaplasia in WTs and p53 mutations, as well as a complete absence of mutations in non-anaplastic tumors. Anaplastic WT is a rare histological subtype with poor prognosis. One of the anaplastic tumors arose in a hereditary setting associated with a cancer-prone family. Our results provide a molecular definition for a distinct subtype of tumor showing poor prognosis and suggest that p53 mutations may be an initiating event for WT development in some familial WTs. (This research was funded by MRC and NCI of Canada. Some of the tumors analyzed in this study were obtained through the Pediatric Oncology Group, Buffalo, N.Y.).

N 501 LOSS OF WILD TYPE C-MYC IN BURKITT'S LYMPHOMAS. Bhatia K, Spangler G, Huppi K, Siwarski D, and Magrath I, Pediatric Branch and Molecular Genetics Laboratory, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

The induction of apoptosis by c-*myc* is a function that at first sight appears to be in opposition to existing data that support only a dominant role for c-*myc* in oncogenesis. This apparent paradox would be readily resolved if the divergent functions of c-*myc* were separable, i.e if some modifications of the protein structure were to result in abrogation of the apoptosis-promoting function of c-*myc* without affecting its ability to induce proliferation.We have screened the entire coding region of c-*myc* (exons 2 and 3), in 57 Burkitt's lymphoma samples (41 tumor biopsies and 16 cell lines) for mutations and find that: 1) sixty five percent of the BLs tested carry at least one mutation in the c-myc protein which cluster in a region spanning amino acids 33-45 or 57-63; 2) the mutations were homozygous in all cell lines and two tumor biopsies, implying that the mutations occur before *myc*/Ig translocation and 3) these results are consistent with the possibility that c-*myc* may function as a recessive oncogene as well as a dominant oncogene, and suggest that mutations in its transactivation domain may have an important role in the pathogenesis of BL, and possibly other tumors.

N 502 A COMPARATIVE STUDY OF PCR-SSCP,SEQUENCING AND IMMUNOCYTOCHEMISTRY, IN THE DETECTION OF P53 MUTATIONS IN COLORECTAL CANCER, K. J. Cripps, S. White, K. Komine, P. J. Carder, C. A. Purdie, A. H. Wyllie, and C. C. Bird.. CRC Laboratories, Department of Pathology, University Medical School, Edinburgh. Scotland

The p53 tumour suppressor gene is commonly mutated in human malignancies, including approximately 60% of colorectal adenocarcinomas. Point mutations of p53 have been shown to stabilise the protein to a level detectable by immunocytochemistry (ICC). In contrast, wild type protein usually has a very short half-life and is therefore undetectable by ICC, thereby allowing the preferential detection of mutated p53 in routine immunocytochemical analysis of tumours. Recent work has suggested however, that mutation and stabilisation of p53 may not correlate completely, since stabilisation of non-mutated p53 can occur, and a subset of mutations exist that fail to stabilise the protein to a level detectable by ICC.

To determine the significance of ICC in the detection of p53 mutations in colorectal adenocarcinomas, we compared in 55 tumours the results of ICC using the antibody Pab1801 with direct evidence for mutation. Mutations were screened for by PCR-SSCP on exons 5, 6, 7, and 8, and confirmed by direct sequencing.

Twenty-seven (27) tumours were scored positive for ICC staining with Pab1801, of which thirteen (48%) were shown to carry a mutation in exons 5-8 by SSCP, and eighteen (18) had mutations by sequencing. Twenty-eight (28) ICC negative tumours revealed five (18%) with a mutation in this region as detected by SSCP. All SSCP detected mutations were confirmed by direct sequencing, resulting in an efficiency for SSCP in detecting mutations of 72%. This gave an overall figure of 66% of ICC+ cases containing a mutation in p53. The remaining 34% of cases suggest either mutations lie outwith the region studied, or that stabilisation of p53 must have occured by some other mechanism.

N 503 SEQUENCE BASED DIAGNOSIS OF RETINOBLASTOMA James M. Dunn, Ph.D., Homa Mostachfe, Ph.D., Qi Jia, MD, Daisy Du, MD, Greg Dee, BASc., Pierre Sevigny, Ph.D., and Brenda L. Gallie, MD, Eye Research Institute of Canada and the Canadian Genetic Diseases Network, Toronto, Canada.

Retinoblastoma (RB) is a rare retinal cancer of infants caused by a mutation affecting the tumor suppressor gene, *RB1*. The majority of heritable retinoblastoma develop because of a new germline *RB1* mutation; infants are diagnosed only when one or both eyes are beyond conventional therapy and must be removed. If tumors are diagnosed when small, treatment saves lives and prevents blindness. Without knowledge of the mutation, all infant sibs and first cousins are examined under general anesthetic seven times in the first three years of life to find small tumors. Once the precise *RB1* mutation in the child is identified, a simple blood test identifies the infants who really need the clinical examinations, while the other children need no special care. We have initiated a major program to move mutation identification from the research lab into the clinic. The mutations responsible for RB fall into three broad classes: small deletions, insertions and/or rearrangements, missense or nonsense point mutations, and large deletions and translocations. The detection for each of these classes requires a different technique. Initial screening by quantitative PCR of each exon, detects large and small deletions and insertions. The exact size and quantity of the product is determined by virtue of a fluorescently tagged primer used in the PCR. Samples with mutations undetected by this screen have each of their 27 exons and the promoter region sequenced. Both these screens are performed on the A. L. F. Sequencer and the data is analyzed using NEXTSTEP software designed in our lab. Only translocations will be missed using these screens and will be detected by FISH with probes flanking *RB1*. The strategy is being optimized and databases are being designed to efficiently handle the large amounts of data collected. The techniques and software being developed will be applicable to any genetic disease locus, particularly tumor suppressor genes with a high proportion of new mutations.

N 504 MOLECULAR ANALYSIS OF FAMILIAL SOFT TISSUE SARCOMA KINDREDS: EVIDENCE FOR p53 INVOLVEMENT AND GENETIC HETEROGENEITY, Armandina Garza[1], Li Shu-Chuan Cheng[2], Michael A. Tainsky[3], Louise C. Strong[2], and Marc F. Hansen[1]. [1]Department of Molecular Genetics, [2]Experimental Pediatrics, and [3]Tumor Biology, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030.

We conducted a systematic survey of cancer in relatives of 159 patients who had been diagnosed with soft tissue sarcoma before age 16 and who had survived at least three years after diagnosis. From the statistical analysis of this cohort, we found strong evidence for an autosomal dominant cancer predisposing gene, although there is evidence for heterogeneity in risk as well. Nine families from the cohort with the highest evidence of a dominant gene were identified and targeted for analysis. The statistically defined genetic predisposition to cancer in these families was associated with high risk of cancer at many sites, with the highest relative risks observed for sites of soft tissue, bone, and a diversity of other sites including prostate, uterine, cervix, melanomas, breast and leukemias and brain tumors with a high risk of second malignant neoplasm in probands and relatives. Germline mutations of the p53 gene have been previously associated with predisposition to Li-Fraumeni syndrome, a cancer syndrome associated with soft tissue sarcomas, breast cancer, leukemia and brain tumors. To test whether germline mutations in the p53 gene were the predisposing events in the nine soft tissue sarcoma families, we amplified DNA from blood and fixed tissue samples of the probands or nearest affected relatives of the nine families for each exon of the p53 gene by polymerase chain reaction. Analysis was by single strand conformational analysis and thermal gradient gel electrophoresis analysis. We found germline mutations in five out of nine kindred samples. The mutations were single base changes located in exons 5, 7 and 8. Each mutation was confirmed by sequencing both sense and antisense strands. Probands from four families showed no evidence of p53 mutations. When linkage analysis was done in one of the families in which no mutation was detected, the analysis failed to show evidence of linkage to either the p53 locus or to chromosome 17p. Thus our data suggest that while inheritance of a germline mutation in p53 is a common diathetic agent in familial sarcoma syndrome, there is genetic heterogeneity in a significant number of kindreds.

N 505 EVIDENCE FOR, AND PRELIMINARY SUBLOCALISATION OF A CHROMOSOME 11 TUMOR-SUPPRESSOR GENE INVOLVED IN UTERINE CERVICAL CANCERS, Garret M. Hampton, Laura A. Penny, Rebecca A. Baergen, Amy Larson, Cheryl Brewer, Su Liao, R. M. Camille Busby-Earle, Andrew Williams, Michael Steel, Colin Bird, Eric J. Stanbridge and Glen A. Evans, Molecular Genetics Laboratory, The Salk Institute for Biological Studies, P.O. Box 85800, San Diego, CA 92186-5800.

Cell biological evidence has suggested a role for a tumor suppressor gene or genes on human chromosome 11 in cervical cancer progression. Specifically, introduction of whole chromosome 11 into cervical tumor-derived cell lines, such as HeLa and SiHa, can suppress the tumorigenicity of these cells implanted in athymic nude mice (1,2). Re-expression of the malignant phenotype in rare segregants has been specifically correlated with loss of part or all of the introduced 'normal' chromosome 11. Consistently, it appears that genetic material on the long (q) arm is eliminated from the cell, somewhat localizing the gene or gene(s) responsible (3). In support of these observations, loss of heterozygosity (LOH) on chromosome 11 has been reported in 6 of 15 matched tumor-normal pairs with two tumors demonstrating LOH for alleles specific to 11q (4). On the basis of this evidence, we have carried out a systematic molecular genetic analysis of chromosome 11 in 33 cervical cancer patients. Sixteen highly polymorphic DNA markers, analyzed by Southern blotting and the Polymerase Chain Reaction (PCR), were used to compare matched DNA samples from constitutional tissue and portions of tumor highly enriched for neoplastic cells. All 33 patients were heterozygous in the constitutional DNA for 8 or more markers distributed on both arms of the chromosome. Of the 33 tumors analyzed, at least 15 (45%) demonstrated loss of polymorphic alleles (Loss of heterozygosity or 'LOH'). Of the genetic events leading to LOH, 8 were confined to the long arm (53%). Assuming that each of the genetic events specific to 11q affect one 'target' locus, the likely position of the gene is 11q22-q24, based on the minimal regions of deletion overlap. Additional matched tumor-normal pairs are being used to further define the minimal region likely to harbor this gene(s).

(1). Saxon, P. J., *et al.* (1986). *EMBO J.*, **5**: 3461-3466.
(2). Oshimura, M., *et al.* (1990). *J. Cell. Biochem.*, **42**: 135-142.
(3). Misra, B. C., *et al.* (1989). *Amer. J. Human Genet.*, **45**: 565-577.
(4). Srivatsan, E. S.*et al.* (1991). *Am. J. Hum. Genet.* **49**: 868-877.

N 506 DIRECT SOLID-PHASE DNA SEQUENCING ANALYSIS OF THE HUMAN p53 GENE, *IN VITRO* AMPLIFIED FROM BIOPSY SAMPLES.

Anders Hedrum, Fredrik Pontén, Joakim Lundeberg and Mathias Uhlén.
Department of Biochemistry, Royal Institute of Technology, S-100 44 Stockholm, Sweden. Department of Patology, University Hospital, S-751 85 Uppsala, Sweden.

Recent developments in molecular genetics has made a more thorough investigation of cancer possible. Techniques for analysing genetic abnormalities have confirmed the presumed presence of mutations in many cancers. In a variety of human tumors, mutations of the p53 gene appears to be the most common genetic alteration. Analysis of these mutations can perhaps provide information about etiology, monoclonal origin or multifocal and prognosis. Here we describe an approach for solid-phase DNA sequencing of the human p53 gene. The samples are basal cell carcinoma isolated from excised human skin tumor tissue and amplified in a nested configuration to increase specificity and sensitivity. In paralell sections are routinely stained for diagnosis and also stained immunohistochemically. Exons 4-9, containing the major mutational hot spots, were analyzed in an integrated protocol for solid-phase DNA sequencing using a robotic work station involving magnetic separation of DNA and analysis of the sequencing products by electrophoresis with automated detection of the fluorescent labelled fragments. The sequencing output is easy to interpret for both homozygous and heterozygous samples.

N 507 ISOLATION OF cDNA CLONES ENCODED IN A REGION WHICH HAS UNDERGONE A HOMOZYGOUS DELETION OF CHROMOSOME 3p IN HUMAN SMALL CELL LUNG CANCER, Charles H. Hensel[1], Maria C. Daly[1], Rui-Hua Xiang[1], Klaas Kok[2], Charles Buys[2], Harry Drabkin[3], and Susan L. Naylor[1], [1]Dept. of Cellular and Structural Biology, Univ. of Texas Health Science Center, San Antonio, TX 78284-7762, [2]Dept. of Medical Genetics, University of Groningen, Groningen, The Netherlands, [3]Division of Medical Oncology, Univ. of Colorado Medical Center, Denver, CO 80262

In human small cell lung cancer (SCLC), at least three distinct genetic loci containing putative or proven tumor suppressor genes contribute to tumor formation and/or progression. Two of these harbor genes encoding the known tumor suppressor proteins pRB and p53. The third locus, on chromosome 3p, possesses demonstrated tumor suppressor activity, but the gene(s) involved has not yet been cloned.

We have isolated P1 genomic DNA clones from a region shown to have undergone a homozygous deletion of chromosome 3p in a SCLC tumor cell line. Using these clones to screen cDNA libraries by conventional or hybrid-selection methodologies, we have isolated several cDNAs from human lung and fetal brain cDNA libraries derived from the region of chromosome 3p harboring the putative tumor suppressor gene. One of these cDNAs encodes a gene, GNAI2B, known to lie within the homozygous deletion. Of 354 independent cDNA clones isolated using a cDNA selection technique, 16 were found to be GNAI2B cDNAs. The GNAI2 cDNA is expressed at low levels in all SCLC cell lines which we have examined excluding the line in which GNAI2 has been deleted. We also have isolated several novel cDNA clones from this region. Characterization of the expression patterns and genomic structures of these novel cDNA clones currently is underway.

N 508 P53 MUTATIONS, AMPLIFICATION OF THE MDM2 GENE, AND INFECTION OF HPV IN JAPANESE ESOPHAGEAL CANCERS, Kanji Ishizaki, Ichio Shibagaki, Takashi Wagata, and Masayuki Imamura, Radiation Biology Center and First Department of Surgery, Faculty of Medicine, Kyoto University, Sakyoku, Kyoto 606, Japan

We have previously analyzed mutations of the p53 genes in Japanese esophageal squamous cell carcinomas and found mutations in 47% of tumor tissues. Since it was reported that mutations of the p53 gene were detected even at a very early stage of esophageal cancers, it is supposed that mutation of the p53 gene is important in development of esophageal tumor cells. However, in our data, only a half of tumor tissues exhibited mutations of the p53 gene, and we suppose that another half tumors should have other genetic changes equivalent with p53 mutations in a regulation of cell proliferation. The product of MDM2 gene and E6 protein of HPV are known to bind to and sequester p53 protein. In some tumors amplification of the MDM2 gene or integration of HPV into genome DNA are supposed to be very important in tumorigenesis causing loss of the normal function of the p53 gene. To know whether MDM2 gene amplification and/or integration of HPV are observed in esophageal tumor cells, we have analyzed our tumor samples. In 36 tumors, 3 cases exhibited amplification of the MDM2 gene and 11 were infected with HPV. The cases with MDM2 amplification were all without p53 mutation and 9 of 11 HPV positive cases were also without p53 mutations. Further more, MDM2 amplification and HPV integration were observed in separate cases. We are now expanding our study with increased number of tumor samples.

N 509 INCOMPLETE PENETRANCE OF FAMILIAL RETINOBLASTOMA AND BENIGN RETINAL TUMORS ASSOCIATED WITH PARTIAL INACTIVATION OF THE RB PRODUCT
Robert A. Kratzke[1], Annette Hogg[2], Gregory A. Otterson[1], Amy B. Coxon[1], Joseph Geradts[3], John K. Cowell[2], and Frederic J. Kaye[1]
[1] NCI-Navy Medical Oncology Branch, National Cancer Institute, and the Uniformed Services University of the Health Sciences, Bethesda, MD 20889. [2] ICRF, Institute of Child Health, 30 Guilford Street, London, WC1N 1EH. [3] Molecular Oncology, Inc, Gaithersburg, MD 20878.

While familial retinoblastoma has served as the paradigm for the two-hit theory of tumorigenesis and for the concept of the tumor suppressor gene, the etiology of incomplete penetrance of familial retinoblastoma is poorly understood. To address the molecular basis for this phenotype we have studied the functional properties of a mutant *Rb* gene identified in a kindred with incomplete penetrance of familial retinoblastoma and evidence for regressed retinal lesions (retinomas). In contrast to all previously isolated RB mutant proteins, we demonstrated that the mutant product from this kindred retained the wildtype properties of nuclear localization, the ability to undergo hyperphosphorylation *in vivo*, and the capacity to suppress growth of $RB^{(-)}$ cells. Protein binding ("pocket") activity, however, was defective. Binding of this mutant protein to the RB binding proteins E1a, cyclins D1, D2, D3, and RBP1, were all markedly reduced (<5%) or absent. In addition, cyclin D2 mediated phosphorylation of the 661W mutant was diminished while cyclin E mediated phosphorylation of the 661W mutant appeared unaffected. The presence of this unique RB mutant in the germline of obligate carriers with incomplete penetrance and regressed retinal lesions suggests a molecular basis for this phenotype and supports the hypothesis that a minimum "RB threshold" level of protein binding activity is required to suppress tumorigenesis.

N 510 ROLE OF GERMLINE p53 MUTATIONS IN PREDISPOSITION TO FAMILIAL OSTEOSARCOMA.

James Luca[1], Edward D. Lustbader[2], Louise C. Strong[3], and Marc F. Hansen[1]. [1]Department of Molecular Genetics and [3]Experimental Pediatrics, The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030 and [2]Fox Chase Cancer Center, Philadelphia, PA 19111

A systematic survey of cancer in relatives of 382 osteosarcoma patients who were diagnosed with osteosarcoma before age 20 revealed evidence for an autosomal dominant cancer predisposing gene. Segregation analysis identified eight families with significant support for the dominant inheritance model. Osteosarcoma has been linked to alterations in the p53 gene and is the most common secondary cancer in patients with germline p53 mutations. To test whether germline mutations in the p53 gene were the predisposing event in these families, we analyzed probands or nearest affected relatives from four of the eight kindreds. The techniques used for screening the p53 gene were PCR amplification of the individual exons of the p53 gene from DNA from peripheral blood or paraffin-embedded fixed tissue followed by Single Strand Conformational Analysis and Temperature Gradient Gel Electrophoresis to detect any heterozygous variants. Variants suspected to be mutations were confirmed by sequencing. In two of the families, heterozygous variants consistent with germline mutation were identified in exon 10. The first was a C—>T transition located at position 1 of codon 337 resulting in a substitution of the amino acid cysteine for arginine. The second was a C—>A transversion located at position 1 of codon 359 substituting the amino acid threonine for proline. These represent previously undescribed variants in a region of the p53 gene previously not associated with germline or somatic mutations and thus potentially expand the regions of the gene in which germline mutations may predispose to cancer. Interestingly, in the remaining two probands, no p53 mutations were found suggesting that there may be heterogeneity in the underlying genetic predisposition. It is possible that mutations in p53 may not be the major gene identified by the statistical analysis but rather a risk modifying event. These results suggest that the search for the major gene in these kindreds should continue.

N 511 LOW INCIDENCE OF p53 MUTATION AND MDM-2 AMPLIFICATION IN NASOPHARYNGEAL CARCINOMA

Kristinn P. Magnússon, Qian Wang, Li-Fu Hu, Fu Chen, Georg Klein and Klas G. Wiman. Department of Tumor Biology, Karolinska Institute, S-171 77 Stockholm, Sweden.

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy with the highest incidence in Southern China and Southeast Asia. Epstein-Barr virus (EBV) infection and dietary and environmental factors have been associated with the disease. p53 mutation and/or deletion of the wild-type p53 allele is the most common genetic change found in human malignancies. We and others have previously found that p53 is frequently mutated in another EBV associated tumor, Burkitt lymphoma (BL). Thus we were interested in determining whether p53 mutation occurs in NPC as well. A series of 10 primary NPC's from Southern China were screened for p53 mutations by DNA sequencing of exons 4 through 9. While 9 out of the 10 tumors had wild type p53, one had two mutations at codon 285 and 287, resulting in a Glu to Lys in both cases. Overexpression of the cellular MDM-2 protein that has been shown to bind and block the function of p53, has been found in sarcomas. We therefore screened a series of NPC's for amplification of MDM-2 by Southern blot analysis. One out of 20 tumors had an 8-fold amplification of MDM-2. This is the first demonstration of MDM-2 amplification in NPC. Because of the low incidence of p53 mutation and MDM-2 amplification we conclude that inactivation of p53 function is not an important factor in the development of NPC.

N 512 THREE GENETICALLY ALTERED COLON CANCER ONCOGENES ARE PRESENT IN A BENIGN CULTURED COLON ADENOMA, WHICH PROGRESSES TO TUMORIGENICITY AND GROWTH FACTOR INDEPENDENCE WITHOUT INACTIVATING THE P53 TUMOR SUPPRESSOR GENE. Sanford Markowitz, L. Myeroff, J. Lutterbaugh, M. Lyon, E. Zborowska, M. Kochera, and James K. Willson, Department of Medicine, Case Western Reserve University and University Hospitals of Cleveland, Cleveland, OH 44106

We describe the spontaneous progression of VACO-235, a human colon adenoma cell line, to tumorigenicity and growth factor independence via a pathway independent of inactivation of the p53 suppressor gene. VACO-235, established from a benign human colon adenoma, is at early passages nontumorigenic in the nude mouse, unable to grow in soft agar, growth stimulated by serum and EGF, and growth inhibited by TGF-beta. VACO-235 daughter passages 93 and higher have in culture spontaneously progressed to being weakly tumorigenic, but retain all other growth characteristics of VACO-235 early passages. A mouse xenograft from late passage VACO-235 was re-established in culture as the granddaughter cell line, VACO-411. VACO-411 is highly tumorigenic, clones in soft agar, and is unresponsive to serum, EGF, and TGF-beta. Genetic analysis demonstrates that early passage VACO-235 bears a mutant K-*ras* allele, bears only mutant APC alleles, expresses no DCC transcripts, and expresses only wild type p53 transcripts. VACO-411 retains the identical genotype, including still expressing only wild type p53. We conclude first that colonic cells following *ras* mutation, APC mutation, and DCC inactivation remain nontumorigenic and growth factor dependent. We also conclude that full malignant progression involves at least two additional steps, and in VACO-411 has proceeded by a novel pathway not requiring p53 inactivation.

N 513 ROLE AND MUTATIONAL HETEROGENEITY OF THE P53 GENE IN HEPATOCELLULAR CARCINOMA,

Naoshi Nishida[1,2], Yoshihiro Fukuda[1], Kazuwa Nakao[1] and Kanji Ishizaki[2], The 2nd Department of Internal Medicine, Faculty of Medicine[1] and Radiation Biology Center[2], Kyoto University, Yoshida-Konoecho, Sakyo-ku, Kyoto 606, Japan.

The mutational spectrum of the p53 gene was analyzed in 53 hepatocellular carcinomas (HCCs). Somatic mutations of the p53 gene were detected in 17 cases. Among these 17 mutations, 9 were missense mutations; the mutations of the other 8 cases were nonsense mutations, deletions, or mutations at intron-exon junctions.These mutations were found in a wide region stretching from exon 4 to exon 10 without any single mutational hotspot. G:C to T:A transversion were predominant, suggesting the involvement of environmental mutagens in the mutagenesis of the p53 gene in a subset of the HCC cases. Mutation of the p53 gene occurred frequently in advanced tumors, although several tumors in the early stages also showed mutations. A deletion map of chromosome 17 was constructed by using 10 polymorphic probes and was compared with the p53 mutations in each cases. Loss of heterozygosity (LOH) on chromosome 17 was observed in 49% of the cases (24 of 49), and two commonly deleted regions were detected (around the p53 locus and at 17p13.3 to telomere). Sixteen of the 17 cases with p53 gene mutation showed LOH around the p53 locus, and mutations were rare in HCCs without LOH. However, no mutations were detected in 8 cases with LOH on 17p, suggesting the possibility that an unidentified tumor suppressor gene(s) located on 17p may have also been involved in hepatocarcinogenesis.

N 514 MUTATIONS IN THE APC GENE IN TWO DISTINCT CLINICAL PHENOTYPES, Lisa M. Pleasants[1], Laura L. Whitaker[1], Linda D. Howard[2], Patrick M. Lynch[2], Henry T. Lynch[3], and Marc F. Hansen[1], Departments of [1]Molecular Genetics and [2]Medical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030 and [3]Department of Preventitive Medicine and Public Health, Hereditary Cancer Institute, Creighton University, Omaha, NB 68178

Familial Adenomatous Polyposis (FAP) is an autosomal dominant disease characterized by the presence of a hundred or more polyps in the colon. Mutations in the APC gene on chromosome 5q have been linked to inheritance of this disease. Much of the study of the APC gene has focused on its role in FAP families. Mutations which give rise to FAP appear most often to result in truncated proteins suggesting that this syndrome is due to either a dominant negative effect of the truncated protein, or to a haplo-insufficiency effect produced by the absence of one half the normal gene product. If FAP represents the strongest mutant phenotype of the APC gene, then it is possible that weak mutant alleles of the APC gene may manifest other phenotypes. One possibility is the Hereditary Flat Adenoma Syndrome (HFAS), a clinically distinct syndrome that has been genetically linked to the APC locus. To determine if FAP and HFAS represent phenotypic variations of APC mutations, we tested DNA samples from the peripheral blood of FAP and HFAS patients and family members for mutations in the APC gene. Our analysis used PCR to amplify individual exons. This was followed by Temperature Gradient Gel Electrophoresis to detect heterozygous DNA variants. Variants detected by these analyses were then sequenced to determine whether the base changes were consistent with predisposing mutation. We have examined probands from several large FAP and HFAS kindreds and identified variants consistent with predisposing mutations throughout the APC gene suggesting that the clinically distinct phenotypes, FAP and HFAS, both appear to be due to mutations in the APC gene but do not appear to be due to segregation of mutations to different regions of the APC gene.

N 515 MDM2 AMPLIFICATION AND P53 MUTATION IN MALIGNANT FIBROUS HISTIOCYTOMA, Ann H. Reid*, Mark M. Tsai*, Cynthia F. Wright*, David J. Venzon+, and Timothy J. O'Leary*, *Department of Cellular Pathology, Armed Forces Institute of Pathology, Washington, D.C. 20306 and +Biostatistics Section, National Institutes of Health, Bethesda, MD 20892

A set of 52 cases of malignant fibrous histiocytoma (MFH) with mean follow-up of 11.8 years was evaluated for amplification of the mdm2 gene and mutation of the p53 gene. Differential PCR was used to detect mdm2 amplification and p53 deletion, while PCR and cycle sequencing was used to detect point mutation of the p53 gene. All tests were carried out on formalin-fixed, paraffin-embedded tissue samples. Amplification of the mdm2 gene was detected in 15 of 52 cases (29%). The p53 gene was found to contain point mutations in four cases, a one base pair deletion in one case, and was apparently entirely deleted in one case. Overall, 12% of the cases demonstrated p53 abnormalities. No case contained both p53 and mdm2 aberrations, lending support to the theory that mdm2 amplification provides an alternative means of inactivating the p53 tumor suppression pathway. The results were tested for correlation of mdm2 or p53 aberrations with time to recurrence and overall survival. The presence of mdm2 amplification did not make a significant difference in time to recurrence or overall survival, although there was a trend toward shorter time to recurrence in patients with amplification. Time to recurrence and overall survival also were not significantly different in patients with p53 aberrations. When, however, the patents were stratified by histologic grade, patients with p53 aberrations appeared to have shorter overall survival although the number of cases is very small.

N 516 ISOLATION OF PUTATIVE NON-SMALL CELL LUNG CANCER SUPPRESSOR GENES BY A MAGNET-ASSISTED SUBTRACTION HYBRIDISATION TECHNIQUE (MAST), Peter Schraml, Rob Shipman, Marco Colombi and Christian U. Ludwig, Molecular Oncology, Zentrum für Lehre und Forschung, Basel, Switzerland.

A Magnet-Assisted Subtraction-hybridisation Technique (MAST) was established allowing the isolation of cDNA clones that are expressed in normal lung tissue but not expressed in the corresponding NSCLC tissue. This subtraction technique, employing tissue cDNA, represents a significant improvement over existing cDNA-based subtraction hybridisation methods, particularly when the amount of mRNA is limiting, as in tumour tissue.

17 cDNA clones were isolated that showed no or weak expression in the tumour tissue, when compared to the corresponding normal lung tissue. 11 of the 17 cDNA clones are highly homologous to cDNA sequences for the following proteins: Pulmonary surfactant proteins SP-A and SP-B; Receptor for Advanced Glycosylation Endproducts of proteins (RAGE); Calmodulin-like protein; Natural killer gene 5 (NKG5); Matrix Gla protein (MGP); Glutamine synthetase; Ubiquitin; Vascular smooth muscle α-actin; Cytoskeletal β-actin; Vimentin. The remaining 6 cDNA clones may represent parts of still unknown genes since no significant homologies were found. The MAST cDNA clones were derived from a patient who developed squamous adenocarcinoma. Northern blot analysis of normal/tumour RNA from three other NSCLC patients showed that the lack of gene expression was independent of NSCLC type and stage.

N 517 MOLECULAR ANALYSIS OF A REDUCED PENETRANCE RETINOBLASTOMA FAMILY: EVIDENCE FOR A NOVEL SPLICING MUTATION WITHIN RB1, Elizabeth L. Schubert[1], Louise C. Strong[2], and Marc F. Hansen[1], Departments of [1]Molecular Genetics and [2]Experimental Pediatrics, The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030

Mutations in the tumor suppressor gene RB1 have been implicated in the oncogenesis of several tumor types including retinoblastoma, osteosarcoma, small cell lung carcinoma, and breast carcinoma. Most mutations previously identified in the RB1 locus result in truncation or deletion of the protein product and thus are of little use in the analysis of the protein function and regulation. In particular, very little is known about control of the mRNA splicing of RB1. We have analyzed a large retinoblastoma pedigree characterized by an unusual phenotype including late onset, unilateral retinoblastoma and a high frequency of unaffected obligate carriers. We have molecular evidence for a novel splicing mutation within the protein binding 'pocket' region of RB1. This cryptic splice, which is not due to a mutation of either the splice donor or acceptor site, results in a lowered efficiency for normal splicing of exon 21 and the production of a variant, nonfunctional cDNA which lacks exon 21. This reduction in efficiency of splicing appears to be the predisposing trait for retinoblastoma in this family. Our discovery and analysis of this cryptic splice site may offer insight into the molecular regulation of splicing of the RB1 mRNA.

N 518 TUMOR SUPPRESSOR PROTEIN THERAPY OF CANCER, H. Michael Shepard, Canji, Inc., 3030 Science Park Drive, San Diego, CA 92121. Tumorigenesis is associated with alterations in mechanisms that regulate cellular proliferation and differentiation. Alterations in autocrine growth control (1), amplification or activating mutations in growth factor receptors or signal transduction elements (2, 3) and regulators of the cell cycle -- the tumor suppressor genes (4) -- all have defined roles in the process of tumorigenesis and the evolution of malignancy (5). Retinoblastoma (RB) and p53 are the best characterized tumor suppressor genes. Tumor suppressor genes have the ability to suppress malignancy of many tumor cells when reintroduced and expressed in tumor suppressor defective host cell backgrounds. These concepts support the development of therapies that re-introduce normal tumor suppressor function into target tumor cells. Possible options include gene therapy vectors to introduce normal tumor suppressor genes into tumor targets. A possible alternative approach is re-introduction of normal tumor suppressor function via protein replacement or peptidomimetic strategies. We have shown that p110RB, purified from a heterologous expression system, can be added to cells in culture, be taken into these cells, and preferentially suppress the growth of RB-altered tumor cells. This response is time and dose dependent, and is accompanied by accumulation of intact p110RB in the nuclei of target cells. Administration of p110RB can also suppress the growth of RB-altered lung tumor xenografts. These results support the potential utility of RB protein or peptidomimetic therapy for lung cancer and other diseases characterized by altered expression of the retinoblastoma gene.

1. Sporn, M. B. and Roberts, A. B. Nature (Lond.) 313: 745-747, 1985.
2. Bishop, J. M. Science 235: 305-311, 1987.
3. Shepard, H. M. et al. J. Clinical Immunology. 11: 117-127, 1991.
4. Levine, A. J. Annu. Rev. Biochem. 62:623-651, 1993.
5. Nicolson GL: Cancer Res 47: 1473-1487, 1987.

N 519 ALLELOTYPE ANALYSIS OF ESOPHAGEAL SQUAMOUS CELL CARCINOMA, Ichio Shibagaki, Kanji Ishizaki, Yutaka Shimada, Masayuki Imanura, Radiation Biology Center and First Department of Sugery,Faculity of Medicine, Kyoto University, Sakyo-ku,Kyoto 606,Japan

Previously we have shown that allelic loss on chromosome 17p, on which the p53 gene is located, is very frequent and loss of function mutations of p53 gene are closely associated with the tumorigenesis of esophageal cancer. To know whether any other tumor suppressor genes are also included in esophageal cancer, we are now conducting allelotype analysis. 54 polymorphic DNA makers on every nonacrocentric autosomal arm except 21p and 22p, are used and RFLP analysis has been performed with 36 pairs of esophageal squamous cell carcinoma(ESC) and adjacent normal tissue specimen. Frequent LOHs more than 30% of informative cases were observed on chromosomes 3p(34.8%), 5q(52.6%), 6p(31.8%), 8p(35.3%), 9p(35.7%), 9q(30.8%), 11p(41.7%), 13q(47.6%), 17p(55.2%), 17q(33.%), 18q(48. 6%), 19q(30.4%). Among these LOHs on 5q, 13q, 17p and 18q were reported previously, and supposed to be related with APC, Rb, p53 and DCC genes, respectly. However, deletion analysis of the chromosome18 revealed that the common region of the loss does not include the DCC locus. This suggests that possible tumor suppressor gene on 18q other than DCC is involved in esophageal tumors.

N 520 ALLELES OF THE APC GENE: AN ATTENUATED FORM OF FAMILIAL ADENOMATOUS POLYPOSIS COLI (APC), Lisa Spirio[1], Sylviane Olschwang[2], Joanna Groden[3], Gilles Thomas[2], Mark Leppert[1] and Ray White[1]
[1]Department of Human Genetics and the Howard Hughes Medical Institute, University of Utah, Salt Lake City, UT 84112, [2]Institute Curie, Laboratoire de Génétique des Tumeurs, 26, rue d'Ulm-75231, Paris, France, and [3]Department of Molecular Genetics, University of Cincinnati Medical Center, Cincinnati, Ohio 45267
An attenuated and phenotypically variable form of familial adenomatous polyposis coli, AAPC, causes relatively few colonic polyps, but still carries a significant risk of colon cancer. The mutant alleles responsible for this attenuated phenotype have been mapped in several families to the adenomatous polyposis coli (*APC*) locus on human chromosome 5q. Four distinct mutations in the *APC* gene now have been identified in seven AAPC families. These mutations that predict truncation products, either by single base-pair changes or frameshifts, are similar to mutations identified in families with classical APC. However, they differ in that the four mutated sites are located very close to one another and nearer the 5′ end of the *APC* gene than any base substitution or small deletion yet discovered in patients with classical APC. Therefore, we propose that *APC* mutations further downstream impose a more severe phenotype through a dominant negative interaction, while *APC* alleles causing AAPC are recessive "loss-of-function" mutations. Alternatively, 5′-end mutants may produce partially-functional gene products by internal or re-initiation of translation from the mutant mRNAs.

N 521 A COMPLETE GENOME SCAN OF 27 RENAL TUMORS FOR LOSS OF HETEROZYGOSITY WITH SPECIAL REFERENCE TO P53, Kenneth D. Tartof, Tamra L. Goodrow, Richard Greenberg, Jerome Freed, Marilyn Bremer, Sharon Howard, Marianna Shasarenko, and Catherine Bingham, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

Cell cultures have been established from 22 human renal tumors and the corresponding lymphocytes from each patient have been immortalized with Epstein Barr virus. In addition, renal tumor tissue and the corresponding transformed lymphocytes from five other patients have been obtained bringing the total number of paired samples to 27. This collection reflects the full range of pathologies associated with renal cell carcinoma (RCC) including both clear cell and mixed (clear/granular) forms of both non-papillary and papillary carcinomas as well as the rarer oncocytic and cystic types.

We are interested in identifying those chromosomes that may contain a tumor suppressor gene associated with a particular RCC pathology. Towards this end we have exploited CA microsatellite polymorphisms to assay loss of heterozygosity (LOH) in each tumor. All 27 patients were screened for LOH with highly informative CA repeat loci (average 87% informative) for 39 chromosome arms. The 5 nucleolus organizing regions and the X chromosome were not examined. Our results show that 6/27 tumors reveal no LOH for any chromosome arm (93% informative). Significantly, 8/17non-papillary clear cell and mixed tumors both reveal LOH for 3p, an observation consistent with previously published results. There may also be a correlation between LOH on 11q and 21q and papillary tumors. LOH was also observed for chromosomes 6, 8, and 14 but these changes occur in several different tumor types.

Five patients presenting with either papillary or mixed clear/granular non-papillary tumors appear to be trisomic for chromosome 17. Two additional patients show LOH for 17p. Since p53 maps to 17p it was possible that this tumor suppressor gene might be affected. There is little published information regarding p53 mutations in renal tumors we, therefore, sought to identify specific exons that may be mutated in this gene using single strand conformation polymorphisms (SSCP). Our preliminary results may indicate the presence of mutations in exons 5, 6, and 7. Sequencing experiments are in progress to identify these putative alterations.

This research was supported by grants from the Betz Foundation, Council for Tobacco Research U.S.A., Public Health Service (Grant CA-06927), and an appropriation from the Commonwealth of Pennsylvania.

N 522 MOLECULAR CHARACTERIZATION OF A CHORIOCARCINOMA TUMOR SUPPRESSOR GENE, Joseph R. Voland and Christopher Becker, Department of Biology and Cancer Center University of California San Diego La Jolla, California 92093

We have previously reported the cDNA sequence of a transmembrane protein, 721P, expressed on the cell surface of syncytiotropohblast, endothelium, and activated lymphocytes and have shown that it contains a cytoplasmic domain homologous to the *myc* family of oncogenes. This similarity includes a leucine zipper, and basic region approximately 60% homologous to the leucine zipper and basic region of *myc*. (Voland *et al.*, *Proc Nat Acad Sci* 89:10425, 1992).

We have transfected this gene, under the control of a CMV promoter/enhancer, into the choriocarcinoma cell lines BeWo, JEG and JaR, as well as the fibrosarcoma line HT1080. The transfected cells show a reduced proliferative capacity, a decreased mitotic index, and fail to produce tumors in nude mice. Mutational analysis of the cDNA shows that removal of the putative basic region results not only in a loss of the "tumor suppressor" effect, but also in an increase in proliferation of the cells.

We have also utilized an epitope tagging method in which an an epitope of the influenza virus was added to the carboxyl terminus of the cytoplasmic domain of 721P. Preliminary immunohistochemical analysis of transfected cells indicates that the epitope appears to associate with the nuclear membrane, suggesting that at least a portion of 721P could translocate to the nucleus.

These results suggest that the 721P protein may play an important role in the differentiation of several cell types, and that the regions of the molecule homologous to *myc* are important in the function of this molecule. Further mutational analysis and biochemical studies are currently underway to better localize this protein in the cell.

N 523 ASSOCIATION BETWEEN P53 MUTATION AND P-GLYCOPROTEIN EXPRESSION IN BONE AND SOFT TISSUE SARCOMAS, Bun-ichiro Wadayama, Junya Toguchida, Yoshihiko Kotoura and Takao Yamamuro, Department of Orthopaedic Surgery, Kyoto University School of Medicine, Kyoto 606, Japan

The function of the p53 protein in normal cells has not yet been clarified. Recently, however, its activity as a transcriptional regulator was shown in some human genes including the multi-drug-resistance gene, MDR1, encoding P-glycoprotein which plays an important role in the mechanism of drug resistance. In this study, the relationship between p53 gene mutation and expression of P-glycoprotein was analyzed immunohistochemically in various types of bone and soft tissue sarcomas. Of 113 tumors analyzed, 28 (24.8%) showed positive staining for the p53 protein. These included 18 of 67 osteosarcomas, 5 of 20 chondrosarcomas, 4 of 11 malignant fibrous histiocytomas (MFHs) and one Ewing's sarcoma. The expression of P-glycoprotein was also analyzed in 86 of these tumors, and positive staining was found in 27 cases (31.4%); 16 of 55 osteosarcomas, 6 of 18 chondrosarcomas, 3 of 8 MFHs, and one case each of malignant lymphoma and Ewing's sarcoma. Strong correlation was found between the expression of p53 and that of P-glycoprotein; 59.3% of p53-positive tumors expressed P-glycoprotein, whereas P-glycoprotein was detected in 18.6% of p53-negative tumors, the difference being statistically significant ($p=0.0004$). Close association was much prominent when only the cases with a missense mutation were considered (7 of 9 cases, 77.8%). These data indicate that p53 mutations, especially missense mutations, may closely associate with P-glycoprotein expression *in vivo*, resulting in the tolerance for chemotherapeutic agents.

N 524 **TUMOR SUPRESSOR GENE THERAPY OF CANCER: ADENOVIRAL MEDIATED GENE TRANSFER OF p53 AND RETINOBLASTOMA cDNA INTO HUMAN TUMOR CELL LINES.** Kenneth N. Wills, Daniel C. Maneval, Patricia Menzel, Suganto Sutjipto, Shu Fen Wen, Karen Nared-Hood, Matthew P. Harris, Whei Mei Huang, Mei-Ting Vaillancourt, Wendy Hancock, Mia Moulton, Margarita Nodelman and Richard J. Gregory. Canji Inc, 3030 Science Park Rd., San Diego, CA 92121.

Mutations or loss of the p53 and/or retinoblastoma (Rb) tumor suppressor genes are associated with a vast array of human malignancies. Re-introduction of wild-type p53 or Rb into these deficient tumor cells has been shown to suppress their tumorigenic properties, and in some cases, induce apoptosis. Therefore, p53 and Rb gene therapy may be a viable means of treating many types of cancer. To address this, we have constructed a series of adenovirus vectors which direct the expression of either wild-type p53 or Rb. These vectors are deleted for the adenoviral E1a and E1b genes required for viral replication and have substituted in their place expression cassettes in which either tumor suppressor genes are driven by the Ad 2 major late promoter or the CMV promoter. An additional construct in which the Rb gene is driven by its own promoter has also been constructed. Infection of p53 or Rb null/mutant tumor cell lines with these viruses indicate that they can express p53 or Rb, suppress DNA replication, affect cell growth and induce apoptosis in some cases. Our results suggest that adenovirus mediated tumor suppressor gene transfer may be an effective treatment for certain types of cancer. We are currently testing this hypothesis in animal models.

N 525 HETEROGENEITY OF RB1 GENE EXPRESSION IN OSTEOSARCOMA, Jiaxiang Zhao[1], Hong-Ji Xu[2], Shi-Xue Hu[2], A. Kevin Raymond[3], Robert S. Benjamin[4], William F. Benedict[2], and Marc F. Hansen[1], Departments of [1]Molecular Genetics, [3]Pathology, and [4]Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030 and [2]The Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX.

Osteosarcoma is the most common primary malignancy of bone in children and adolescents. The current classification of osteosarcoma is based on three general criteria: differentiation of the tumor cells, type of matrix produced by the tumor cells, and anatomical location of the tumor. Osteosarcoma can be broadly divided into two histological categories based on incidence: conventional osteosarcoma and variant histology osteosarcoma. Loss of heterozygosity and functional inactivation of the retinoblastoma susceptibility (RB1) gene is a common feature in conventional histology osteosarcoma occurring in 75% or more of tumors. Well differentiated intraosseus osteosarcoma is one of the variant histology osteosarcomas with a distinct clinical behavior from conventional histology osteosarcoma. Analysis of well differentiated intraosseus osteosarcomas revealed no loss of heterozygosity at the RB1 gene. Further analysis by in situ immunohistochemistry revealed normal RB1 protein expression suggesting that these tumors arise by a different genetic mechanism than conventional histology osteosarcoma.