MOLECULAR BASIS OF CANCER THERAPY

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Molecular Basis of Cancer Therapy

Oncogenes and Tumor Suppressors: Cellular Approaches

R 001 REGULATION AND FUNCTION OF RAS PROTEINS IN CELLULAR GROWTH AND TRANSFORMATION, Julian Downward, Patricia H.Warne, Pablo Rodriguez-Viciana, Sydonia Rayter, Tanya Basu, Bengt Hallberg and László Buday, Signal Transduction Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, U.K.

Ras proteins are key components of signal transduction pathways linking extracellular stimuli to cellular growth and differentiation responses. Ras proteins function as biological switches, being active when in the GTP bound state and inactive when bound to GDP. They possess intrinsic GTPase activity which is regulated by the GAP family of proteins, including $p120^{GAP}$ and neurofibromin, the product of the *NF1* gene. GAPs negatively regulate the Ras proteins and in some cases are themselves regulated, for example by phorbol esters in T cells. A more widely used mechanism for the regulation of Ras is the control of guanine nucleotide exchange rates by the exchange factor Sos. This protein binds to activated receptors, such as the EGF receptor, through the SH2/SH3 containing adaptor protein Grb2. A number of other Grb2 binding proteins have been characterised, including Shc, an SH2 protein that is phosphorylated on tyrosine in response to most of the stimuli that are known to activate Ras and may be important in its regulation. Mechanistic details of the control of Sos exchange activity towards ras will be discussed.
Events downstream of Ras are likely to be of great significance in the design of drugs to target tumours involving activating mutations of Ras, some 30% of all human malignancies. Ras is now known to interact directly with the serine/threonine kinase Raf, which is likely to be a major effector of Ras. The ability of Ras to regulate Raf kinase activity has not yet been demonstrated; it appears that other components are required for this interaction to influence the enzymatic activity of Raf. An alternative explanation is that the regulation of Raf by Ras is entirely at the level of moving the kinase from the cytosol to the plasma membrane where its critical targets might be located, although this would appear to exclude the MAP kinase pathway, the only known downstream target for Raf, which is cytosolic. Interaction of Ras and Raf can be demonstrated in intact cells in response to stimuli which activate Ras.
A number of other possible downstream targets of Ras may exist apart from Raf. These will be discussed.

Oncogenes and Tumor Suppressors: Genome Approaches

R 002 THE MOLECULAR GENETICS OF MULTIPLE ENDOCRINE NEOPLASIA TYPE 2 (MEN 2), Bruce Ponder, Lois Mulligan, John Kwok, Charis Eng, Alan Tunnacliffe, Margaret Ponder, Emily Gardner, Julie Moore, Kate Healey, Mark Elsdon, CRC Human Cancer Genetics Research Group, Dept. Pathology, University of Cambridge, Tennis Court Rd, Cambridge CB2 1QP, UK.

MEN 2 is a dominantly inherited cancer syndrome in which developmental abnormalities are associated with tumour development. The tissues primarily involved are the C-cells of the thyroid, the adrenal medulla and the enteric nervous system (which are derived from neural ectoderm) and the parathyroids, which are derived from the endoderm of the 3rd and 4th branchial arches.

There are three varieties of MEN 2 which differ in the spectrum of tissues involved. Germline mutations in the ret protooncogene, a receptor tyrosine kinase which maps to 10q11.2, have been found in the commonest form, MEN 2A (which affects thyroid, adrenal and parathyroid) and in F-MTC (familial medullary thyroid carcinoma: thyroid only); but not so far in MEN 2B (thyroid, adrenal but not parathyroid, with enteric nervous system abnormalities), nor in sporadic thyroid or adrenal tumours.

The mutations in the ret protooncogene in MEN 2A and F-MTC affect one of five cysteine residues in a cysteine-rich region in the extracellular domain of the protein. There is a correlation between both the site and the precise type of the mutation, and the spectrum of tissue involvement. The mutated gene is probably dominant at the cellular level, in which case it is the first example of germline activation of an oncogene in inherited cancer.

Interestingly, in view of the involvement of the enteric nervous system in MEN 2B, a gene for Hirschsprungs disease (an inherited absence of intrinsic enteric ganglion cells) also maps to the MEN 2 region. The search for the MEN 2B and Hirschsprungs mutations is in progress.

Mechanisms of Cell Killing: Apoptosis

R 003 EXAMINATION OF THE ROLE OF p53 IN TUMORIGENESIS, APOPTOSIS, AND RESPONSE TO ANTICANCER AGENTS. Scott W. Lowe[1], Earlene M. Schmitt[1], H. Earl Ruley[2], David E. Housman[1] andTyler Jacks[1]. [1]Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139. [2]Department of Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, TN 37232

We have constructed a mouse strain carrying a targeted disruption of the p53 tumor suppressor gene. This strain has allowed the investigation of the role of p53 in suppressing tumorigenesis in the whole animal, and cells derived from these animals have been used to examine p53 function in vitro. We have concluded from experiments carried out in vivo that p53 function is not required for cellular viability or differentiation, since p53-deficient animals survive gestation normally. However, absence of p53 function greatly accelerates tumorigenesis; 100% of p53 homozygous mutant mice develop tumors by nine months of age. Heterozygosity for the p53 mutation also predisposes to tumor formation, although with longer latency.

Experiments carried out in vitro using p53-deficient cells (as well as heterozygous and wild-type controls) have demonstrated a requirement for p53 in the normal cellular response to DNA damage. Specifically, fibroblasts lacking p53 do not arrest in the G1 phase of the cell cycle following gamma-irradiation. Also, immature thymocytes lacking p53 fail to undergo programmed cell death (apoptosis) in response to ionizing radiation. This latter result may account for the high frequency of thymic lymphoma in p53-deficient mice. These data support the "guardian of the genome" hypothesis for p53 function.

p53-dependent apoptosis is also important to transformation in other circumstances. Cells expressing the adenovirus E1A oncogene are highly prone to apoptosis, particularly when placed in growth restricting conditions, and this death can be suppressed by coexpression of the adenovirus E1B gene. We have shown that p53-deficient fibroblasts are resistant to E1A-induced apoptosis and, in fact, can be transformed to a tumorigenetic state by the expression of this single oncogene. Finally, apoptosis of E1A-expressing cells can be induced by a variety of anticancer agents, including radiation and several chemotherapeutic drugs. Efficient apoptosis of oncogene-expressing cells is dependent on p53 function, since p53-deficient cells expressing E1A are highly resistant to these treatments. Thus, the mutation of p53 can accelerate the tumorigenesis process and generate cells which are cross-resistant to a number of anticancer treatments. These data suggest that p53 status of tumors may be an important determinant of the efficacy of cancer therapy.

Mechanisms of Drug Resistance

R 004 MECHANISMS OF MULTIDRUG RESISTANCE, Piet Borst[1], Alfred H. Schinkel[1], Frank Baas[2], Jaap J.M. Smit[1], Lisette W.H.M. Eijdems[1], Guido J.R. Zaman[1], and Sander J. Smith[1], [1]Division of Molecular Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands, [2]Department of Neurology, Academic Medical Center, Amsterdam, The Netherlands.

Within the field of multidrug resistance, we are mainly concentrating on two topics and we shall present updates on the ongoing work.
Topic one is the phenotype of mice with a disrupted P-glycoprotein (Pgp) gene. We have generated mice homozygous for a disruption in mdr1 (mdr1b), mdr2, or mdr3 (mdr1a). The mutations do not interfere with viability or fertility showing that these Pgps have no indispensable role in early development or metabolism. Mice homozygous for a disruption of mdr2, however, develop liver disease and this appears to be due to their complete inability to secrete phospholipids into bile (1). This suggests that the mdr2 Pgp (and its human MDR3 homologue) is essential for translocating phospholipids through the hepatocyte canalicular membrane in which it is located. The phospholipid translocator function of this Pgp may not be commensurate with drug transport, explaining why this Pgp is not involved in MDR. Mice homozygous for the mdr1a disruption are hypersensitive to some drugs. These mice will be useful in assessing long-term (side-) effects of effective reversal agents, that block Pgp function in MDR tumors.
The second topic is MDR in the human non-SCLC line SW-1573. When these cells are selected in vitro with low levels of doxorubicin or vincristine, they usually develop a form of non-Pgp MDR with decreased drug accumulation (2) that can be reversed by genistein (3). The MDR1 mRNA level is decreased (rather than increased) in these mutants (4) and the MRP mRNA levels are usually unaltered (5). This raises the possibility that neither MDR1 nor MRP overexpression is responsible for MDR in these cells, but a third gene, as yet unidentified.

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R 005 MULTI DRUG RESISTANCE MEDIATED BY MRP, A NOVEL ATP-BINDING CASSETTE TRANSPORTER, Roger G. Deeley, Caroline E. Grant, Kurt C. Almquist, David R. Hipfner, Gunnar Valdimarsson, Ebba U. Kurz, Brenda Stride, Douglas W. Loe and Susan P. C. Cole, Cancer Research Laboratories, Queen's University, Kingston, Ontario, Canada.

Increased expression of P-glycoprotein (P-gp) has been shown to be the cause of multidrug resistance in many *in vitro* drug-selected cell lines and has been implicated in the drug resistance of tumors *in vivo*. However, in some major forms of malignancy, such as lung cancer, multidrug resistance is a common occurrence but overexpression of P-gp is rarely observed. A number of multidrug resistant cell lines have also been characterized which do not overexpress P-gp. Multidrug resistance associated protein (MRP) was cloned recently from such a cell line, H69AR, that was derived by selection in doxorubicin from the human small cell lung cancer cell line, H69. Although H69AR cells are resistant to a spectrum of natural product drugs similar to that which results from overexpression of P-gp, resistance is not reversed by agents such as cyclosporin A.

Overexpression of MRP mRNA has now been detected in multidrug resistant cell lines derived *in vitro* from cancers of the cervix and breast, small and non small cell lung tumors, fibrosarcoma and leukemias. The major form of MRP mRNA encodes a protein with a predicted M_r of 171 kD that contains two nucleotide binding folds (NBFs), several potential glycosylation sites and 12 transmembrane regions, divided 8 and 4 between the NH_2- and COOH-proximal halves of the molecule, respectively. Although MRP and the P-glycoproteins are members of the same superfamily of proteins, primary sequence homology is low and this is limited primarily to the generally conserved regions of their predicted ATP binding domains. Amino acid identity is less than 15%. Several splicing variants of MRP mRNA have also been detected which encode proteins with predicted deletions in NBFs and transmembrane regions.

We have confirmed that MRP is an ATP binding, integral membrane, 190kD-glycophosphoprotein and that it is capable of conferring multidrug resistance when transfected into HeLa cells. The results of these and ongoing experiments designed to determine the mechanism by which MRP confers resistance, the functional significance of various splicing variants and its possible physiological role will be discussed.
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R 006 MURINE P-GLYCOPROTEIN: IDENTIFICATION OF PHOSPHORYLATION SITES, Susan Band Horwitz, Chia-Ping H. Yang and George A. Orr, Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461

Drug resistance constitutes a major problem in the treatment of human malignancies. Evidence is accumulating that multidrug resistance is one form of drug resistance that has a role in human tumors. The multidrug resistance phenotype is associated with the overproduction of P-glycoprotein, an integral membrane phosphoprotein that acts as an energy-dependent drug efflux pump with a broad specificity for hydrophobic antitumor drugs. Each half of P-glycoprotein is designated as a cassette that contains six putative transmembrane domains. Each cassette is followed by a nucleotide binding domain and the two cassettes are joined by a linker region. P-glycoprotein is phosphorylated in cells and it has been suggested that phosphorylation may regulate the drug transport activity of P-glycoprotein. Using cyanogen bromide digestion and immunoblot analysis, the major phosphorylation sites in mdr1b P-glycoprotein have been mapped within amino acid residues 627-682 in the linker region. Serine 669 has been identified as the single protein kinase C phosphorylation site in the linker region by FABMS analysis and two dimensional phosphopeptide mapping. The in vitro protein kinase A phosphorylation site was identified as serine 681 by site-directed mutagenesis. The linker region of P-glycoprotein is encoded by a single exon, is highly charged with alternating acidic and basic side chains, and contains several consensus phosphorylation sites. These characteristics fit the operational definition of the cystic fibrosis conductance regulator R domain whose phosphorylation modulates chloride transport activity. The effects of phosphorylation on P-glycoprotein function are being evaluated.

Metastasis, Angiogenesis and More

R 007 ACQUIRED 'MULTICELLULAR' DRUG RESISTANCE OF SOLID TUMORS AND ITS RELATIONSHIP TO CANCER METASTASIS, Robert S. Kerbel, Sunnybrook Health Science Centre, and Dept. of Medical Biophysics, University of Toronto, Toronto, Ont., Canada.

The Problem: Expression of drug resistance and competence to metastasize are among the most significant impediments to the treatment of advanced cancers. These two properties are generally studied as separate entities. However, there is a growing body of evidence to suggest that there is a functional linkage between the two. For example, transfection of cell lines with certain oncogenes can endow the recipients with increased intrinsic resistance to a variety of chemotherapeutic drugs (reviewed by Kerbel, *J. Cell. Bioch.*, in press, 1994). Hence, the same genetic alterations which 'drive' tumor progression — and the metastatic process — may also be responsible, in part, for aspects of intrinsic (or acquired) drug resistance. If so, one might anticipate that tumor sublines selected for drug resistance would express a more aggressive ability to metastasize. In fact, the opposite result is generally observed when drug-resistant sublines, previously selected *in vitro*, are grown *in vivo*. Another problem associated with cell lines selected for resistance *in vitro* is the prolonged periods of time usually required for their selection, in comparison to the relatively rapid development of drug resistance in the clinical setting. Both of these problems could conceivably be circumvented by the isolation and study of resistant sublines selected *in vivo*.
The Results: In 1990 Teicher et al. reported details of the isolation *in vivo* of a number of alkylating agent resistant sublines from the EMT-6 mouse mammary carcinoma (*Science*, 244:1457-61, 1990). The sublines expressed their resistance properties only *in vivo*, and not *in vitro*. Analysis of these sublines revealed they expressed a remarkably enhanced ability to metastasize to the lungs after subcutaneous or mammary fat pad inoculation of the cells.
A New Paradigm for Acquired Drug Resistance? Subsequent studies in our laboratory revealed that the drug resistance properties of the EMT-6 sublines could be fully restored *in vitro*, provided the cells were grown as multicellular tumor 'spheroids:' growth as conventional monolayers, or disaggregation of intact spheroids, resulted in complete or significant loss of resistance, respectively (Kobayashi et al., *P.N.A.S.* 90:3294-98, 1993). Moreover, the structure of the drug-resistant spheroids was far more compact than the parental cell population. Thus this type of acquired drug resistance is different from all other known or suggested mechanisms in that it is not expressed at the single cell level. More recent experiments have revealed that this type of multicellular drug resistance, (but not unicellular resistance) including changes in spheroid structure, can be rapidly induced in a variety of human or mouse cancer cell lines by a single transient exposure to chemotherapeutic drugs. These observations may provide an explanation for the ability of solid tumors to frequently develop resistance in an accelerated manner in the clinical situation. The molecular and cellular basis of multicellular forms of drug resistance is currently under investigation in our laboratory.

Receptors and Antigens as Targets for Therapy

R 008 THE INTERLEUKIN-2 RECEPTOR: A TARGET FOR IMMUNOTHERAPY OF LEUKEMIA/LYMPHOMA, Thomas A. Waldmann, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Human T-cell lymphotropic virus I is a retrovirus associated with tropical spastic paraparesis (TSP), infectious dermatitis, polyarthritis, interstitial pneumonitis and uveitis. Furthermore, it is associated with an aggressive leukemia, adult T-cell leukemia (ATL). There is no standard curative chemotherapy for ATL that on mean is lethal within 9 months. The leukemic cells of patients with HTLV-I-associated ATL express the IL-2 receptor α, β and γ subunits. In contrast, normal resting cells do not express the IL-2 receptor α-subunit identified by the anti-Tac monoclonal antibody. Patients with ATL were treated with different forms of IL-2 receptor-directed therapy to exploit the difference in IL-2 receptor expression between normal and malignant cells. Using the unmodified anti-Tac monoclonal antibody, one-third of the 19 patients with ATL treated have undergone a remission, in two cases complete. There was no toxicity observed. However, unmodified murine monoclonal antibodies are limited by their immunogenicity and their poor effector functions. To address these issues we used genetic engineering to produce humanized anti-Tac. This humanized monoclonal antibody contains the complementarity-determining regions from the mouse with the remainder of the antibody derived from human IgG1-κ. Humanized anti-Tac is dramatically less immunogenic than the murine version, has improved pharmacokinetics and, in contrast to the parent antibody, manifests antibody dependent cellular cytotoxicity with human mononuclear cells. To enhance its effector function anti-Tac was armed with toxins or with α- and β-emitting radionuclides. In a clinical trial with ^{90}Y-anti-Tac at the doses used (5, 10 and 15 mCi), 12 of the 18 patients with ATL underwent a partial or sustained complete remission. A new trial has been initiated using humanized anti-Tac armed with Yttrium-90. Thus, the clinical application of IL-2 receptor-directed therapy provides a new perspective for the treatment of certain neoplastic diseases including HTLV-I-associated ATL.

Clinical Trials of Novel Cancer Therapies

R 009 REVERSAL OF MULTIDRUG RESISTANCE (MDR) WITH CHEMOSENSITIZERS: AN EMERGING CLINICAL REALITY, Sydney E. Salmon, Thomas P. Miller, Thomas Grogan, Alan List and William S. Dalton, Arizona Cancer Center, University of Arizona College of Medicine, Tucson AZ.

Acquired drug resistance is a reason for treatment failure in patients (pts) with Multiple Myeloma (MM), Malignant Lymphoma (ML), and Acute Myeloid Leukemia (AML). We have observed that only a 2-3% of untreated MM or ML pts' tumors express MDR due to P-glycoprotein (p-170) immunohistochemically, whereas up to 60% of drug resistant pts with these disorders are p-170+. However, p-170 positivity of AML is often seen in previously untreated pts. We've also found that increased P-glycoprotein (p-170) expression by tumor cells correlates with in vitro resistance to adriamycin (A) and vincristine (V) in the human tumor cloning assay (HTCA). A number of drugs have been identified that can modulate P-glycoprotein and act as chemosensitizers. Verapamil (Ve), Quinine (Q) and Cyclosporin (Cy) were selected because these agents exhibit chemosensitizing activity on p-170+ human tumor cell lines at clinically achievable doses. To evaluate chemosensitizers clinically, we administered the VAD regimen (D=dexamethasone) with the addition of high dose Ve (VAD/Ve) to 22 pts with MM who failed VADalone. Five of these pts (23%) achieved second remissions with VAD/Ve). For patients with ML in relapse from multiple agents, CVAD/Ve (C=cyclophosphamide) was used. Out of 18 ML pts treated in relapse, 8 (44%) achieved PRs and 5 (28%) achieved CRs, for an overall response rate of 73%. Finally, in drug resistant variants of AML, we found a high response rate to ARA-C/Daunorubicin plus Cy. In myeloma and lymphoma, response was more frequent in p-170+ patients. However, in the AML category, this was not always the case. For a series of AML patients, MDR message expression was followed with quantitative PCR both prior to therapy and at the time of relapse from treatment with ARA-C/Daunorubicin plus Cy. Of interest, MDR expression was markedly reduced at relapse suggesting that a new form of resistance emerged in MDR negative clones. These laboratory and clinical observations suggest that a new approach to drug resistance may be emerging. Accordingly, we believe that further evaluation of chemosensitizers is warranted to reverse MDR in hematologic neoplasms and possibly other forms of p-170+ cancer as well.

Cell Cycle; Oncogenes and Tumor Suppressors: Cellular Approaches

R 100 OVER-EXPRESSION OF p53 AND HIGHER PROLIFERATIVE IN VITRO RESPONSES TO SAC + IL-2 STIMULATION CORRELATE WITH CLINICAL PROGRESSION IN B-CLL. Aguilar-Santelises M., Magnusson K., Wiman K., Mellstedt H. and Jondal M. Departments of Immunology and Tumor Biology, Karolinska Institute, and Department of Oncology, Karolinska Hospital, Stockholm, Sweden.

The p53 status of leukemic cells from B-CLL patients, non progressive and progressive, has been analyzed by cell ELISA, immunoprecipitation, FACS and cDNA sequencing together with *in vitro* proliferation in response to *Staphylococcus Aureus* strain Cowan 1 (SAC) and IL-2. In FACS, cells from progressive leukemia were found to over-express p53 which was recognized by the monoclonal antibodies PAb 240 and PAb 1801. In a PAb 240 based cell ELISA, 60% of progressive B-CLL samples were positive. However, when cDNA from five of these, corresponding to exons 5 to 9, was sequenced, only one mutation was found (codon 179). Among progressive B-CLL populations, 10/14 responded by proliferation *in vitro* to SAC + IL-2. In non-progressive B-CLL cells, low levels of p53 were found by FACS, none were positive in the PAb 240 cell ELISA and only one case showed a weak proliferative response to SAC + IL-2. Clinical progression is associated with a higher *in vitro* proliferative capacity of the leukemic cells. Over-expression of the p53 protein may contribute to the progressiveness of the disease but p53 mutations have not been found often involved.

R 101 REGULATION OF PROLIFERATION IN THE NEW HODGKIN CELL LINE HD-MYZ BY ANTISENSE-OLIGONUCLEOTIDES AGAINST IL- 6 AND IL- 8

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We have recently described a novel Hodgkin cell line HD-MyZ, which displays a myelo-monocytic phenotype. This cell line expresses constitutively transcripts for IL-1β, IL-6 and IL-8 and secretes large amounts of these cytokines into the supernatant. In addition HD-MyZ cells constitutively express mRNA's for IL-1α, IL-5, IL-7, IL-10, IL-1 receptor (type I) and IL-6 receptor. Stimulation of cells with PMA increases mRNA expression as well as the secretion of IL-1β, IL-6, IL-8 and induces *de-novo* expression of IL-8 receptors.

Since HD-MyZ cells constitutively express IL-1β and IL-6 as well as their receptors these cytokines might act as autocrine growth factors for this cell line. We studied the effects of IL-1β, IL-6 and IL-8 antisense-oligonucleotides on the protein secretion and the spontaneous proliferation of HD-MyZ cells.

Interestingly, protein secretion and proliferation of HD-MyZ cells could be specifically inhibited *in vitro* by 3'modified phosphothioate antisense-oligonucleotides (AS) against IL-6, IL-1β and IL-8. In contrast sense- or nonsense-oligonucleotides did not have any effect on protein-secretion or proliferation. Xenotransplantation of HD-MyZ cells into SCID mice by intravenous or subcutaneous inoculation led to development of disseminated tumors with infiltrative and destructive growth. In addition lymphadenopathy, pleural effusion and infiltration of spleen were observed. Our SCID mice model might prove helpful in developing new therapeutic strategies *in vivo*.

R 102 PRECLINICAL STUDIES OF IRON CHELATION CANCER THERAPY FOR NEUROBLASTOMA. Christopher N. Frantz, Moira Lawrence, Kelly Stone, Allen E. Eskenazi, Patricia E. Berg, Marcello Sztein, The Children's Cancer Foundation Laboratory, Department of Pediatrics, University of Maryland School of Medicine, Baltimore, MD 21201.

Some neuroblastomas are unusually sensitive to the cytotoxic effects of iron chelation. Clinical investigations of the chelator desferoxamine (DFO) in children with neuroblastoma are underway. Expression of the N-myc oncogene is believed to play a role in the malignant behavior of neuroblastoma. We recently demonstrated in cultured LA-N-1 human neuroblastoma cells that incubation with DFO resulted in 1) decreased expression of N-myc occurring at the level of transcription, 2) increased expression of transferrin receptors, presumably due to a decrease in cellular iron caused by the chelator, 3) extension of neurites suggesting cellular differentiation, 4) cell cycle arrest in both G1 and S phase, and then 5) cell death. We now report that DFO and other iron chelators decrease expression of N-myc in all 8 human neuroblastoma cell lines studied. However, neurite extension occurred in only 3/8 cell lines. In addition, prolonged incubation of LA-N-1 cells with low concentrations of DFO and other chelators resulted in decreased N-myc expression, S phase arrest, and eventual cell death. In contrast, higher chelator concentrations were required for neurite extension and G1 arrest. We conclude that iron chelation may result in cell death without inducing G1 phase cell cycle arrest. Also, the decrease in N-myc expression induced by iron chelation is not due merely to G1 arrest. Future experiments will address the sensitivity of neuroblastoma to the cytotoxic effects of iron chelation and its relation to N-myc expression.

R 103 INDUCTION OF GADD153 PROMOTER ACTIVITY BY DDP AND TAXOL PROCEEDS THROUGH SEPARATE PATHWAYS. Gately, D.P. and Howell, S.B. Dept. of Biomedical Sciences and Cancer Center 0812, University of California, San Diego, La Jolla, CA 92093-0812

Gadd153 is a "growth arrest and DNA damage-inducible gene" that is induced by a number of anti-cancer agents including cisplatin (DDP) and taxol. The GADD153 protein is structurally related to the CCAAT/enhancer-binding protein (C/EBP). A common translocation in myxoid liposarcoma fuses a glutamine-rich region (similar to that found in the SP-1 transcription factor) to the full-length GADD153 protein. We have found that gadd153 promoter activity and gadd153 mRNA are induced to the same extent by taxol treatment. However, gadd153 promoter activity is weakly induced compared to the induction of gadd153 mRNA after equal doses of DDP. We have also found that a 1 hr pre-incubation with the tyrosine kinase inhibitor tyrophostin B46 (Tyr B46) inhibits the ability of DDP to activate the gadd153 promoter. However, this treatment has no effect on the ability of taxol to activate the gadd153 promoter. The effect of Tyr B46 on the toxicity of DDP and taxol was investigated by colony-forming assay. Tyr B46 decreased the IC_{50} of a 1 hr DDP treatment from 5.0 μM to 2.5 μM. The combination of these two drugs was found to be synergistic by median effect analysis. Try B46 also decreased the IC_{50} of a 24 hr taxol treatment from 5.1 nM to 2.4 nM. We hypothesize that the DNA damage induced by DDP treatment activates a protective pathway in the cell that includes a Tyr B46-inhibitable tyrosine kinase upstream of gadd153 promoter activation. Although this Tyr B46-inhibitable protective pathway is also activated by taxol treatment, taxol treatment activates a separate pathway which is responsible for the activation of gadd153 promoter activity. This second pathway is not activated by treatment with DDP.

R 104 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.

R 105 INDUCTION OF TERMINAL DIFFERENTIATION OF A MEGAKARYOCYTIC LEUKEMIA CELL LINE WITH IL-3 AND RIBAVIRIN

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The megakaryocytic leukemia cells, CMK, have been shown to express some megakaryocytic markers and morphological features following phorbol ester treatment. We have developed a new induction protocol which involves long term treatment of the cells with the combination of IL-3 and the nucleoside analogue ribavirin. During treatment the cells express both early and late megakaryocytic markers as well as morphological features which are similar to those displayed by primary megakaryocytes. These include a change in ploidy and the formation of alpha granules and a demarcation membrane system. mRNA for late markers such as PF4, beta thromboglobulin, GMP140, and thrombospondin, show synergy between the two inducers which is only evident after 6 days of treatment. The proliferative capacity of cells after two week treatment is greatly reduced relative to controls. This treatment protocol may provided an approach to studying the induction of differentiation and the reduction of proliferation of these leukemia cells.

R 106 p53 EXHIBITS A DEPHOSPHORYLATION DEPENDENT CONFORMATIONAL CHANGE DURING LYMPHOCYTE ACTIVATION, John McClure, Christine Noonan, and William Shearer Dept. of Pediatrics, Baylor College of Medicine, Houston TX 77030.

Tumor suppressor p53 can activate transcription of genes that negatively regulate the cell cycle. The multiply-phosphorylated protein appears to be temporally and spatially regulated by its state of phosphorylation. In order to study regulation of p53 function during lymphocyte activation, we developed a sensitive competition RIA utilizing synthetic peptide representing conserved Domain V of p53 and anti-Domain V antiserum. Immunoaffinity purified p53 from T cells produced dose reponse curves that were parallel to those produced by peptide standard. Suspecting that C-terminal Domain V might be a conformationally masked epitope, we incubated purified p53 with enzymes, both phosphatases and kinases, and observed that immunoreactive p53 (IRp53) increased 3-5 fold upon treatment with alkaline phosphatase or PP2A but not with PP2B. Treatments with kinases, particularly with $p34^{cdc-2}$ kinase or casein kinase II, produced IRp53 levels that were 20-70% lower than untreated control level. Domain V was, therefore, an epitope that was masked by phosphorylation (decreased IRp53) and unmasked by dephosphorylation (increased IRp53). When Raji cells were treated with PMA/A23187, cytosolic IRp53 rose 50-150% above control levels within 10-20min and then declined to control level by 60min. Nuclear IRp53 declined by 50-120% with a minimum at 10-20min, returned to control level by 70-90min, and exceeded control level at > 90min. The same events were observed in peripheral blood T cells stimulated with mitogens Concanavalin A / serum. Staurosporin (100nM) had little effect on increased cytosolic IRp53 but abrogated the decline of nuclear IRp53. Okadaic acid (1nM) totally inhibited the increase of cytosolic IRp53 and enhanced the decline of nuclear IRp53 levels by 30-70%. We concluded that cytosolic p53 was mobilized and dephosphorylated, probably by PP2A, during lymphocyte activation. We propose that the nuclear localization signal sequence of p53 may be coordinately unmasked with Domain V by dephosphorylation during cell activation and that this process precedes its translocation to the nucleus where p53 can act upon genes that modulate the cellular response to positive growth signals. An understanding of mechanisms that regulate the nuclear functions of p53 will permit the rational design of therapeutic agents that are targeted to the restoration of normal cell growth control.

R 107 SELECTIVE INHIBITION OF RAS-DEPENDENT CELL TRANSFORMATION BY INHIBITORS OF FARNESYL PROTEIN TRANSFERASE, A. Oliff, N.E. Kohl, S.L. Graham, G.D. Hartman, and J.B. Gibbs, Departments of Cancer Research and Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486

Ras genes harboring oncogenic mutations are found in approximately 25% of all human cancers and are believed to contribute to the transformed phenotype of these tumors. The oncoprotein encoded by these genes is initially synthesized as a cytoplasmic precursor which requires posttranslational processing to attain biological activity. The critical biochemical alteration that activates the Ras precursor is farnesylation of the cysteine residue present in the CAAX motif located at the carboxy-terminus of all Ras proteins. Once farnesylated and further modified the mature Ras protein is inserted into the plasma membrane where it participates in the signal transduction pathways that control cell growth and differentiation. The farnesylation reaction that modifies Ras is catalyzed by a specific enzyme termed farnesyl-protein transferase (FPTase). We have prepared inhibitors of this enzyme in an effort to identify compounds that would block Ras-induced cell transformation and thereby function as Ras-specific anticancer agents. A variety of natural products and synthetic organic compounds that specifically inhibit FPTase activity were isolated. Several of these compounds were found to block farnesylation of Ras proteins in cell culture, and were able to block the growth of Ras-transformed cell lines in tumor colony forming assays. FPTase inhibitors also blocked the morphologic alterations associated with Ras-induced transformation of mammalian cell lines. By contrast, these compounds did not affect the growth or morphology of cells transformed by the Raf for Mos oncogene proteins which do not require farnesylation to achieve biological activity. Whole animal pharmacology and antitumor activity studies employing these FPTase inhibitors are in progress. Results from these studies will be discussed.

R 108 EXOGENOUS WT-p53 EXPRESSION DOES NOT AFFECT THE COLONY FORMING EFFICENCY OF NORMAL BONE MARROW CELLS, Silvia Soddu, Raffaella Scardigli, Giovanni Blandino, Marco Crescenzi and Ada Sacchi, Molecular Oncogenesis Laboratory, Regina Elena Cancer Institute, 00158 Rome, Italy.

Some gene therapy approaches for cancer treatment attempt to transduce tumor suppressor genes into tumor cells. A problem central to this strategy is the functional targeting of tumor cells while avoiding damage to normal cells. It has been observed that introduction of exogenous wt-p53 into non-transformed, wt-p53 expressing cells (normal rat embryo fibroblast, VACO 235 adenoma cells) causes no observable effects. In contrast, transduction of normal p53 into tumor cells that have lost their normal alleles or bear p53 mutations induces various effects that range from growth arrest to apoptosis or differentiation. In this view, a complete purging of the bone marrow from leukemic cells bearing an altered p53 gene can be achieved *in vitro* by delivering wt-p53 to all of the marrow cells. We undertook a series of experiments to demonstrate that transduction of wt-p53 into normal cells is not harmful. In a pilot study on the untransformed myeloid precursor 32D cells, we found that overexpression of the wt-p53 gene caused no phenotypic changes and no reduction of the proliferative rate. Subsequently, human wt-p53 cDNA was cloned into the Babe vectors, that carry the selectable marker for puromycin resistence, and transfected in the ΩE packaging cells, that produce ecotropic viruses. Normal bone marrow cells were infected by co-cultivation with mitomycin-treated packaging cells. The colony forming efficiency of these infected bone marrow cells was tested by *in vitro* cultivation in the presence of IL-3, GM-CSF, or EPO, and puromycin. No difference was found between cells infected with the retrovirus carrying the wt-p53 gene and those infected with the control viruses, that express only the selectable marker.

R 109 FGFR4 AND PLK, TWO PROTEIN KINASE GENES, WHICH SHOW ALTERED EXPESSION IN HUMAN NEOPLASMS, MIGHT SERVE AS MOLECULAR MARKERS FOR HUMAN LUNG TUMORS

Klaus Strebhardt, Uwe Holtrich, Thomas Karn, Andreas Bräuninger, Beatrix Böhme, Georg Wolf, Andreas Doermer and Helga Rübsamen Waigmann, Georg-Speyer-Haus, Paul Ehrlich-Str. 42-44, 60552 Frankfurt, Germany

Screening of a variety of human adult tissues has shown that expression of the FGFR4-gene is restricted to lung, liver and kidney. The analysis of more than 150 lung tumors of different histological subtypes has shown that the expression of FGFR4 is markedly reduced compared to surrounding lung tissues.

Furthermore, we have identified a new serine/threonine kinase -gene PLK (polo-like kinase). The mRNA expression of PLK appears to be strongly correlated with the mitotic activity of cells: Resting peripheral lymphocytes do not express the gene at all. When primary T-cells are activated by phytohaemagglutinin (PHA), a high level of PLK transcripts results within 2-3 days. In some cases exogenous addition of IL-2 to these cells increases the expression of PLK mRNA further. In line with a function of PLK mRNA expression in mitotically active cells is also our finding that six immortalized cell lines examined expressed the gene. In A431 cells this expression was down-regulated by serum starvation and enhanced after serum was added again.

Tumors of various origin (lung, colon, stomach, smooth muscle and esophagus as well as Non-Hodgkin lymphomas) expressed high levels of PLK transcripts in about 80 % of the samples studied, while PLK mRNA was absent in surrounding normal tissue, except for colon. The only normal tissues where PLK mRNA expression was observed so far were colon and placenta, both known to be mitotically active. No PLK transcripts were found in normal adult lung, brain, heart, liver, kidney, sceletal muscle and pancreas.

Taken together, we suggest that the combined analysis of PLK- and FGFR4-expression of sputum specimens could improve current diagnostic and therapeutic procedures for human lung tumors.

R 110 INHIBITION OF EGR AND E2F FAMILIES IN NEOPLASTIC BREAST CELLS, Renee J. Szakaly, Jill E. Slansky, and Peggy J. Farnham, Department of Oncology, University of Wisconsin, Madison, WI 53706

Progression of cells through the cell cycle is controlled by interconnected signal transduction pathways which cause the temporary activation or repression of growth-responsive genes. Work from many laboratories suggests that various signal transduction pathways are constitutively activated in tumor cells. We are testing if inactivation of two distinct families of growth-responsive transcription factors can affect the growth of neoplastic breast cells. The EGR family of genes encodes proteins with three zinc fingers and includes EGR1, EGR2, EGR3, and EGR4. mRNA levels of this family can be increased by both growth factors and activated oncogenes suggesting that these proteins may be involved in the transcriptional regulation of cell cycle pathways. We have shown that EGRs 1, 2, and 4 differentially regulate the *egr2* promoter. Our results suggest that the EGR family members do not simply encode redundant proteins, but instead may each mediate specific responses to serum growth factors. The E2F family of genes encodes proteins with possible helix-loop-helix DNA binding domains and includes E2F1, E2F2, E2F3, E2F4, DP1, and DP2. We have demonstrated that not all promoters which contain consensus E2F sites are activated by E2F1, again suggesting that the members of the E2F family have specific functions. To determine what effects the members of these families have in the growth of neoplastic breast cells, we are inhibiting the function of EGR and E2F family members in neoplastic breast cells by using dominant negative constructs and specific ribozymes. If we can inhibit the growth of neoplastic breast cells, we will have identified a potential target for therapy.

Oncogenes and Tumor Suppressors: Genome Approaches; Transcription and Translation; Signal Transduction

R 200 OVER-EXPRESSION OF N-MYC INHIBITS THE EXPRESSION OF FIBRONECTIN AND ITS RECEPTOR, THE INTEGRIN α5β1. Birkenmeier, T. M., O'Brien, S., and Boedeker, E., Department of Medicine, Washington University Scool of Medicine, St. Louis, MO.

Over-expression of the myc family of oncogenes is a common occurrence in small cell lung cancer. However little is known about the relationship between oncogenesis and myc gene amplification. Because alterations in the interaction of cells with the extracellular matrix is a frequent occurrence during oncogenesis, we investigated the effects of N-myc over-expression on the expression of two molecules involved in cell adhesion, the integrin α5β1 and its extracellular matrix ligand, fibronectin. We have discovered that over-expression of N-myc inhibits expression of the α5β1 and fibronectin. For the α5 gene this occurs at the level of gene transcription and appears to be due to effects on the interaction of the transcription factors NF-κB and NF-1 with their DNA binding sites located in the α5 gene promoter. In addition, we have found that treatment of a small cell lung cancer cell line with bromodeoxyuridine results in a loss of the amplified N-myc gene with subsequent expression of α5β1 and FN. The cells that express the α5β1 integrin exhibit a marked decrease in their ability to form colonies in soft agar or tumors in nude mice as compared to the cells that do not express this integrin. We propose that alterations in the expression of cell adhesion molecules as a result of myc gene over-expression is an important factor in oncogenesis.

R 201 EXPRESSION OF THE HUMAN KREV-1 TUMOUR SUPPRESSOR GENE IN THE LUNGS OF TRANSGENIC MICE

S. Damak[1], P.M.George[2] and D.W.Bullock[1]. [1]Centre for Molecular Biology, Lincoln University, Canterbury, New Zealand [2]Clinical Biochemistry, Christchurch Hospital, New Zealand.

Mice of the A/J strain are useful models of lung cancer because they develop tumours spontaneously or after treatment with ethyl carbamate. Many human lung tumours involve K-ras activation. Transformation with K-ras can be reversed by co-expression of the Krev-1 gene in cultured cells. To test the tumour suppressor activity of Krev-1 *in-vivo*, we have produced transgenic mice by microinjection of a human Krev-1 cDNA under the control of the rabbit uteroglobin (UG) promoter, which directs expression specifically to the lung. A genomic DNA fragment containing 3.3 Kb of UG promoter region (UG 3.3) was ligated to the complete Krev-1 cDNA and microinjected both into A/J and Swiss Random White (SRW) mouse zygotes, resulting in 7 lines of SRW and 2 lines of A/J transgenic mice. A Northern blot of total RNA probed with a Krev-1 probe showed no over-expression in the lungs of transgenic mice. To assess possible tumour suppression, mice from line 1803 (A/J background) were given ethyl carbamate to induce lung tumours. The mean number of lung tumours obtained in the transgenic group was lower than in the non-transgenic group (22.1 ± 1.2 versus 26.1 ± 1.2, $n=9$, $p < 0.05$). This tumour suppressor effect may be due to weak expression of human Krev-1 that was undetectable by Northern blotting. Experiments to demonstrate such expression using RNAse Protection Assay are in progress. To obtain higher Krev-1 mRNA expression in the lung, we have made a new construct consisting of a mutated Krev-1 cDNA, where Gln in codon 63 was replaced by Glu as this has been shown to have a more potent tumour suppressor activity in tissue culture. The mutated construct was cloned into exon I of a human neutrophil elastase minigene, which we have shown previously gives high pulmonary expression under the control of UG 3.3 and 3 lines of transgenic mice were produced. Krev-1 mRNA expression in the lung of these mice will be discussed.

(Supported by a grant from the NZ Cancer Society).

R 202 THE USE OF ALLELE LOSS TO DEFINE NEW TUMOUR SUPPRESSOR GENES IN LYMPHOMA, **Morgan GJ, Randerson J, Quirke P, Lewis F. Department of molecular pathology, Algernon Firth institute, University of Leeds, Leeds, W Yorkshire, UK.**

The description of CA repeats and a method for their detection using the PCR has enabled the generation of high resolution maps of the genome using these loci to be defined. These were primarily derived in order to provide an anchor map for further genetic cloning. In addition to this they provide a means by which it is possible to search the genome of patients with a malignancy for sites of consistent allele loss. Sites consistently lost in specific tumours may be assumed to be associated with tumour suppressor genes which may prove to be potential targets for therapy in the future. The PCR primers can be used to isolate further DNA fragments which will contain the gene of interest which can be further characterised. Using radioactive methods for the detection of these CA repeats is time consuming and lacks the accuracy necessary for the consistent detection of the loss of one allele. We have therefore used fluorescent PCR primers and the ABI genescanner equipment as both a way of circumventing these problems and as a means to allow multiplexing of primers so that several loci can be examined simultaneously.

We are in the process of using this technology to examine low grade lymphomas. Rather than randomly screen the genome we have adopted an approach of screening potentially interesting loci first. In lymphoma such loci include 1q21-23, 1p32-36, 2, 3q, 5q, 6q21-25, 7q32, 8p, 9q31-34, 11q23, 17, Xp and Y. Initially we are focusing on chromosome six and will present data using 13 CA repeat loci from this chromosome. Using different colour primers and CA repeats of different sizes it should be possible to look at many sites in a single lane greatly facilitating the screening process, to date we have be able to multiplex up to five loci and analyse them in a single lane.

R 203 ALLELIC DELETION, MUTATION AND EXPRESSION OF PUTATIVE TUMOUR SUPPRESSOR GENES IN HUMAN NON-SMALL CELL LUNG CARCINOMA, Rob Shipman and Christian Ludwig, Molecular Oncology, University Hospital Research Center (ZLF), Basel, CH-4031

Allelic deletion on chromosome 11p and 17p was investigated in 61 paired normal lung and primary non-small cell lung carcinoma (NSCLC) samples. Two regions of 11p displayed non-random allelic loss, specifically 11p13 and 11p15. However, the observed allelic deletion was biased toward 11p13. 11p13 contains genetic loci for the catalase gene (CAT), the Wilms' tumour gene (WT1) and the gene for the ß-chain of follicle-stimulating hormone (FSHß). 76% of the NSCLC cases displayed allelic deletion at CAT. Loci telomeric to CAT, including loci at 11p15, showed reduced allelic deletion suggesting that allelic deletion of a gene(s) near CAT may be involved in the progression of NSCLC. YAC clones containing the CAT locus are being examined and prepared for use in a direct selection procedure for cDNAs encoded at this region. Loci immediately centromeric to CAT are also being analysed by VNTR-PCR. Although 38% of the NSCLC cases showed deletion of a WT1 allele, no mutations were detected in the remaining WT1 allele. WT1 expression was also investigated in fetal tissues, normal lung and NSCLC tissue. WT1 expression was observed in fetal lung and hematopoietic tissue and in adult lung and NSCLC. Allelic deletion at 17p13, a region known to contain the p53 gene, immunohistochemical and sequence analysis of the p53 gene were also examined in our NSCLC samples. These analyses support the contention that dysfunctional expression of the p53 gene is a pivotal event in the genesis of NSCLC.

R 204 PHOSPHOROTHIOATE C-*MYC* ANTISENSE OLIGODEOXYNUCLEOTIDE REDUCES VIABILITY, PROLIFERATION, AND C-*MYC* PROTEIN EXPRESSION IN AN HL-60 MODEL, Shirley Williams, Eileen Gillan, Yu Suen, Lori Ishizawa, and Mitchell S. Cairo., Children's Hospital of Orange County, Orange CA 92668

Constitutive c-*myc* expression is associated with Burkitt's lymphoma and selected forms of acute lymphoblastic leukemias (B-ALL) resulting from reciprocal chromosome translocations between c-*myc* on chromosome 8 and immunoglobulin loci on chromosomes 14, 22, or 2. We have been interested in developing an antisense oligodeoxynucleotide (ODN) strategy to inhibit the proliferation of c-*myc*-dependent lymphomas for use as a molecular purging agent in bone marrow transplant therapy. HL-60, a human promyelocytic leukemia cell line, also overexpresses c-*myc* and has become the model system for our current study. Antisense ODN to c-*myc* may inhibit c-*myc*-dependent tumor proliferation and/or induce cellular differentiation. Preliminary studies utilizing an unmodified 15-mer antisense ODN (5'GCA-CAG-CTC-GGG-GGT3') spanning the 5'-cap region of the c-*myc* mRNA (Bacon, *Oncogene Res* 6:13, 1991) have demonstrated tumor inhibition. Our proliferation assays to study the inhibitory effect of the unmodified ODN were performed in 96-well plates with 5000 HL-60 cells in 100 µl RPMI-1640/15% FBS per well. Concentrations as low as 50 µg/ml decreased proliferation of HL-60 cells approximately 40% ($p<0.0001$) as compared to controls. Cell counts and viability were monitored using trypan blue staining after 3-7 days incubation with ODNs. Subsequent experiments utilized a more nuclease-stable, phosphorothioated antisense ODN (5'GCA-GCA-CAG-CTC-GGG-GGT3'). A time-course study revealed a 3-fold inhibition of proliferation of HL-60 cells at 96 and 120 hours with 50 µg/ml antisense. Ten-fold less of the modified antisense (1µg/ml) was required to inhibit 50% (LD_{50}) proliferation of HL-60 cells as compared to the unmodified antisense. A 60% decrease in the native c-*myc* protein level was observed at 100 µg/ml of modified antisense in HL-60 cells, using immunoblot analysis with a mouse monoclonal c-*myc* Ab (Ab-1, Oncogene Science), and a secondary Ab (rabbit anti-mouse IgG) to intensify the signal strength of the tertiary Ab (goat anti-rabbit) conjugated to alkaline phosphatase. Additionally, concentrations up to 10 µg/ml of modified antisense ODN did not decrease CFU-GM formation (145 ± 35%) ($p=0.27$) in human bone marrow, suggesting that these levels of antisense would have a negligible effect on normal cells. These studies suggest that modified c-*myc* antisense ODNs inhibit growth and c-*myc* expression in a c-*myc*-dependent leukemia cell line more effectively than unmodified without toxicity to human bone marrow, and might form the basis for a molecular approach to Burkitt lymphoma therapy as an *ex vivo* bone marrow purging agent.

R 205 TWO GENES, *RCK* AND *MLL/ALL-1/HRX*, ARE INVOLVED IN 11q23 TRANSLOCATION IN HEMATOPOIETIC MALIGNANCIES, Kazuhito Yamamoto[1], Masao Seto[1], Shinsuke Iida[1], Yoshitoyo Kagami[1], Toshitada Takahashi[2], and Ryuzo Ueda[1], Laboratories of [1]Chemotherapy and [2]immunology, Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya, 464 Japan.

Chromosomal translocations involving chromosome 11 band q23 are unique in that this region is translocated with a variety of partner chromosomes and that these abnormalities are associated with various types of hematopoietic malignancies. We cloned two translocation-associated genes from this region. One is the *RCK/p54* gene associated with t(11;14)(q23;q32) involved in a B-cell lymphoma, encoding a 472 or 483 amino acid polypeptide belonging to the translation initiation factor/helicase family. Another is *MLL/ALL-1/HRX* gene involved in infantile leukemias and secondary leukemias. *MLL/ALL-1/HRX* gene product is 3969 amino acid polypeptide, related to *Drosophila* trithorax, containing two putative DNA-binding motifs consisting of three AT-hook motifs and two multiple zinc finger domains. In leukemias with 11q23 translocation, two putative DNA-binding motifs are disrupted and *MLL/ALL-1/HRX* is fused to genes on partner chromosomes, resulting in fused mRNA encoding chimeric proteins. In t(11;19)(q23;p13) translocation, *LTG19* on 19p13, encoding a serine/proline-rich 559 amino acid polypeptide, is involved as a partner gene. By RT-PCR analysis with specific primer pairs, fusion of *MLL/ALL-1/HRX* to *LTG19* was demonstrated in 8 out of 9 leukemias with t(11;19) translocation. Fusion-points of LTG19 to MLL/ALL-1/HRX are at amino acid 5 in most cases and at amino acid 371 in a cell line. Furthermore, the C-terminal portion of LTG19 has high homology to that of LTG9 which is more than 342 amino acid polypeptide on 9p22 involved in t(9;11)(p22;q23) translocation. Thus, the conserved C-terminal portion of LTG9 and LTG19 is speculated to have important functions for leukemogenesis. Leukemogenetic property and potential transcriptional activity of the fusion proteins are now under investigation.

Mechanisms of Cell Killing: Apoptosis; Mechanisms of Drug Resistance

R 300 APOPTOSIS INDUCED BY INHIBITION OF INTERCELLULAR ADHESION Richard C. Bates, Andre Buret, and Gordon F. Burns, Cancer Research Unit, The University of Newcastle, 2308, New South Wales, AUSTRALIA.

The LIM 1863 colon carcinoma cell line grows as structural 'organoids' of goblet and columnar cells around a central lumen and provides a model for the development of stem cells in the normal colon. To determine the adhesion molecules involved in organoid formation we attempted to block this process by single cell suspensions of LIM 1863 reseeded in the presence of monoclonal antibodies. An anti-integrin antibody directed against a conformational epitope on the αv subunit totally inhibited organoid reformation. As a consequence of this inhibition, failure to establish intercellular contacts resulted in a rapid apoptotic response by these tumor cells, an event accompanied by translocation of the tumor suppressor gene product p53 from the cytosol to the nucleus. These findings suggest that cell-cell adhesion may be a vital regulator of colon development overcome in tumor cells by loss of adhesion molecules or of functional p53 protein.

R 301 HOMOZYGOUS p53 MUTATIONS INCREASE THE RESISTANCE OF HUMAN SKIN FIBROBLASTS TO UV-IRRADIATION. James M. Ford and Philip C. Hanawalt, Departments of Oncology and Biological Sciences, Stanford University, Stanford, CA 94305-5020.

The p53 tumor suppressor gene product serves as a cell cycle checkpoint control element, and may regulate the response of mammalian cells to DNA damage by either inducing a cell cycle arrest at G_1/S (presumably to facilitate adequate DNA repair prior to replication), or inducing programmed cell death (apoptosis). Cells with mutant p53 fail to arrest or undergo cell elimination via apoptosis after DNA damage. Thus, mutations of the p53 gene might alter the sensitivity of normal or neoplastic cells to DNA damage as a consequence of less efficient DNA repair, or decreased cell death. To investigate these possibilities, we determined the UV sensitivity of primary human skin fibroblasts from patients with Li-Fraumeni syndrome (LFS), a familial cancer syndrome, containing a single base mutation on one allele of the p53 gene (heterozygous mutants (+/–)), and immortalized sublines expressing only mutant p53 (homozygous mutants (–/–)), having undergone loss of heterozygosity at the p53 gene locus (from M. Tainsky, MD Anderson, TX). The sensitivity of each cell line to UV-irradiation was determined by clonogenic assay. Actively growing cells were plated in triplicate, exposed to 0 - 20 J/m^2 UV-irradiation, and colonies allowed to form for 14 - 21 days. The surviving fraction for each UV dose was calculated, expressed logarithmically, and the initial dose necessary to reduce survival to 10% (D_{10}) derived by linear regression analysis. For each of 2 sets of cells lines, the homozygous p53 mutant cells (–/–) were 2-fold more resistant to UV cytotoxicity than the heterozygous lines (+/–). Analysis of gene specific and overall genomic DNA repair of UV-induced pyrimidine dimers, and the incidence of damage induced apoptosis, are being determined for these cell lines for comparison with control fibroblasts expressing wild-type p53, to elucidate the mechanism of resistance to UV irradiation in homozygous mutant p53 skin fibroblasts.

R 302 EPIGENETIC CHANGES AND COLLATERAL DRUG SENSITIVITY IN CELLS TREATED WITH VERAPAMIL, Bernard W. Futscher[1], Joe Costello[2], Yei-Mei Peng[1], Russel O. Pieper[2], William S. Dalton[1], [1]Arizona Cancer Center, Tucson AZ, 85724, & [2]Loyola University Medical Center, Maywood IL, 60153.

The inclusion of non-toxic chemosensitizers in cancer therapies necessitates understanding the influence that chemosensitizers have on the evolution of the drug resistance phenotype. Following long term exposure to non-toxic concentrations of a chemosensitizer, verapamil, human multiple myeloma cells displayed an increased genomic content of 5-methylcytosine compared to untreated cells. Like the genome overall, the 5' promoter region of the DNA repair gene, O^6-methylguanine DNA methyltransferase (MGMT), was also hypermethylated in the verapamil-treated cells. This hypermethylation of the MGMT promoter was correlated with a decrease in MGMT mRNA and functional MGMT protein. The decrease in functional MGMT resulted in a collateral sensitivity to the chemotherapeutic nitrosoureas. In two mechanistically distinct multidrug resistant cells selected with doxorubicin and verapamil a similar collateral sensitivity to nitrosoureas was observed, and was also accompanied by a loss in MGMT expression and functional protein. The described epigenetic and phenotypic changes in these verapamil-treated cells is stable for at least one year out of drug. Current studies are designed to determine whether this *in vitro* collateral sensitivity is also observed *in vivo*.

R 303 MECHANISMS INVOLVED IN THE SELECTIVE ENHANCEMENT OF VINCA-ALKALOID CYTOTOXICITY BY THE ANTI-EPILEPTIC PHENYTOIN. R. Ganapathi, D. Grabowski, K. Weizer, J.Ford, T. Rice and R. Bukowski. Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44195.

The identification of agents with a large therapeutic index to circumvent clinical antitumor drug resistance is being actively pursued. The anti-epileptic phenytoin (DPH) which inhibits polymerization of tubulin, selectively enhances the cytotoxicity of vincristine (VCR) but not doxorubicin in wild-type or multidrug resistant (MDR) human or murine tumor cells. These effects of DPH on VCR cytotoxicity were not due to binding to P-glycoprotein or increasing VCR accumulation (Cancer Res. 53:3262, 1993). We have determined the interaction of DPH and its congeners on vinca-alkaloid cytotoxicity in murine (L1210/S) and human (promyelocytic leukemia HL-60, non small cell lung carcinoma, NSCLC-3) wild-type or multidrug resistant (L1210/R2, HL-60/DOX 0.1) tumor models to characterize the mechanisms involved in modulation of cytotoxicity. Non-cytotoxic concentrations of 10-20 μg/ml DPH enhanced cell kill induced by VCR , vinblastine (VLB) or vinorelbine (VRL) by 3-15 fold in wild-type and MDR human or murine tumor models. The enhancement of cytotoxicity was: (a) accompanied by a 3- to 5-fold increase in the mitotic index of treated cells; and (b) dependent on continuous treatment with DPH plus VCR for 24 h *and DPH plus* VLB or VRL for 48 h. The major metabolite of DPH, parahydroxy-phenytoin as well as a synthetic congener of DPH which are inactive as anti-epileptics, were as effective as DPH in enhancing VCR or VLB cytotoxicity. In contrast to VLB alone, the combination of VLB plus DPH was also unique in preventing the rapid emergence of VLB resistance in NSCLC-3 cells. Results demonstrate that DPH is a novel agent to enhance the antitumor effects of vinca-alkaloids in the treatment of human malignancies.

Supported by USPHS CA35531

R 304 THERAPEUTIC EFFECTS OF IOR-T1 (ANTI CD6) MURINE MONOCLONAL ANTIBODY IN PATIENTS WITH T-CELL LYMPHOMAS.

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IOR-T1 (IgG2a anti-CD6) monoclonal antibody (mAb) was infused intravenously for a period of two hours, twice a week for two consecutive weeks into ten patients with T cell lymphoma in a phase I clinical trial. The scaling levels of antibody were 200(50 mg/inf.), 400(100mg/inf.), and 800mg(200mg/inf.). The clinical response was evaluated according to the WHO recomendations for measurable malignant skin lesions. Eigth/ten (80%) patients had objective clinical response. At the 200mg dose level, 2/2 had complete response (CR) of 6 and 8 months of duration without disease (WD); at the 400mg dose level 2/4 had CR of 5 and 6 months WD and 2/4 had partial response (PR) of 3 and 2 months of duration; at the 800mg dose level no patient (n=4) received the total dose because of side effects. The doses given were 500, 675, 700 and 730mg. The clinical response was PR (2 months), CR (7 months WD), progression and stabilization respectively. The side effects observed were: fever (80%), trembles (20%), headache (20%), hypertension (20%), hypotension (10%), malaise (10%), vomiting (10%), allergic rash (10%), bronchoespasm (10%) and distal cyanosis (10%). No significant changes in any routine laboratory parameters were observed. Immunohistochemical studies of skin biopsies showed a correlation between a decrease of the CD6 positive phenotype with clinical improvement of the disease and specific in vivo binding of IOR-T1 mAb to the malignant cell. 40% of the patients developed human antimouse antibodies (HAMA) after the 20 days of treatment.

The IOR-T1 mAb recognizes a new epitope distinct from those earlier reported in the CD6 molecule. We report evidence for coupling of CD6 to a protein tyrosine kinase pathway. Crosslinking of the CD6 receptor in combination with TPA cause rapid tyrosine phosphorylation mainly in 70KDa cellular substrate which was inhibited by herbimycin A. Crosslinking induce T cell stimulation, IL-2R upregulation and IL-2 mRNA expression. These events were also inhibited by herbimycin A. It is possible, although not shown, that the signalling capacity of CD6 may be involved in causing regression of T cell lymphoma.

R 305 INDUCTION OF *pgp3* EXPRESSION AND REVERSION OF THE MDR PHENOTYPE IN 9-OH-ELLIPTICINE RESISTANT CELLS TRANSFECTED WITH THE *MYC* ONCOGENE, Alain Jacquemin-Sablon, Charlotte Delaporte and François Dautry, Unité de Biochimie-Enzymologie, Institut Gustave Roussy, 94805 Villejuif, France.

Chinese hamster lung cells resistant to the DNA topoisomerase II inhibitor 9-OH-ellipticine are cross-resistant to various drugs through the expression of the MDR phenotype. A 10-fold amplification and a 20-fold overexpression of the *myc* gene were observed in the parental DC-3F cells as compared to the 9-OH-ellipticine resistant cells. Transfection of the resistant cells with a mouse *c-myc* gene did not alter the resistance to topoisomerase II inhibitors, but reversed the MDR phenotype (Delaporte et al. Exp. Cell Res. 197, 176-182, 1991). Northern and western blot analyses revealed an increased expression of the *pgp1* gene in the 9-OH-ellipticine resistant cells which was not modified in the *myc* transfected clones. However, *myc* expression in these clones resulted in an increased expression of the *pgp3* gene, roughly in proportion of the *myc* expression level. Furthermore, transfection of the human MDR II gene in the DC-3F/9-OH-E cells also resulted in the reversion of the MDR phenotype. From our results, we conclude that (i) in this cellular system, expression of the transfected *myc* gene positively regulates *pgp3* expression but has not effect on *pgp1*; (ii) reversion of the MDR phenotype is proportional to the *myc* and *pgp3* expression levels; (iii) this reversion resulting from the *pgp3* expression is associated with a decreased functional activity of the *pgp1* protein, and was only observed when *pgp1* and *pgp3* were expressed in the same cells with an appropriate stoechiometry.

R 306 **ACQUIRED RESISTANCE TO ANTITUMOR AGENT-INDUCED APOPTOSIS IN HUMAN LEUKEMIA CELLS**, [1]Shiro Kataoka and [1,2]Takashi Tsuruo, *[1]Institute of Molecular and Cellular Biosciences, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan, [2]Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Kami-Ikebukuro, Toshima-ku, Tokyo 170, Japan*

Some antitumor agents kill certain leukemia cells in a process characteristic of apoptosis; although the drugs are known to have different molecular targets. These findings indicate the existence of a common pathway of cell death (apoptosis) induced by drugs. The mechanisms of drug-induced apoptosis, however, are not known yet. To study these mechanisms we isolated a mutant (UK711) with a "apoptosis resistant" phenotype from monocytic leukemia U937 cells by mutagenesis and then screened the cells using etoposide (VP-16) and 1-b-D-arabinofuranosyl-cytosine. The wild-type U937 cells and the mutant UK711 cells showed the similar levels of VP-16-induced DNA strand breaks, as detected by filter elution method. However, U937 and UK711 cells showed substantial different responses to VP-16-induced apoptosis. In addition to VP-16, the mutant UK711 cells showed apoptosis resistance to a variety of antitumor agents, including Ara-C, mitomycin C, camptothecin and adriamycin. The UK711 cells, however, did not show apoptosis resistance to vincristine. The UK711 cells showed apoptosis resistance to cycloheximide, staurosporine, UV irradiation and heat shock as well. These results imply that the mechanism required for apoptosis of the wild-type U937 cells was changed, and the mutant UK711 cells acquired the resistance to apoptosis induced by a variety of stimuli, including antitumor agents.

R 307 NUCLEAR PROTEIN-PROTEIN INTERACTIONS WITH HUMAN DNA TOPOISOMERASE IIα IN HeLa CELLS, David J. Kroll, Division of Pharmaceutical Sciences, University of Colorado School of Pharmacy, Denver, CO 80262

The DNA decatenation activity of type II DNA topoisomerase (topo II) is essential for cellular survival. Therapeutically, topo II has become increasingly important as the target of several, structurally diverse antiproliferative anticancer drugs. Delineating the mechanisms of topo II enzyme regulation may have consequences for maximizing the cytotoxic efficacy of topo II-targetting drugs. A potentially novel mechanism of regulation of topo II by direct protein-protein interactions has been inferred from the presence of a putative leucine zipper in the primary sequence of the α form of the enzyme. We have shown previously that the leucine zipper-containing transcriptional activators CREB, ATF-2, and c-Jun each form protein-protein complexes with a 590 amino acid fragment of the C-terminus of human topo IIα in Far Western assays. CREB's effect on topo II catalytic activity is observed as: 1) a reduction in drug-induced DNA cleavage intermediates and, 2) an increase in overall kDNA decatenation activity. These data suggest that interaction of CREB with topo II shifts the topo II reaction equilibrium to the right. Therefore, topo II-interactive proteins (TIPs) may influence cellular sensitivity to topo II poisons. Our recent work has shown that topo II's putative leucine zipper is not necessary for protein-protein interactions with either itself or transactivating factors. Hence, the goals of the present work are to 1) identify the structural motif in topo II governing these interactions and, 2) characterize other proteins which interact with topo II. Deletion studies have indicated that an amphipathic region encompassing amino acids 857 to 906 of human topo IIα is sufficient for protein-protein interactions. To identify novel TIPs, cytosol/plasma membrane and nuclear extracts were prepared from exponentially proliferating and quiescent cultures of HeLa cells and TIPs detected by Far Western protein binding assays using [^{125}I]topo II (857-906) as the probe. At least twelve HeLa TIPs were observed with molecular masses ranging from 30 to 231 kDa. All TIPs were specifically found in nuclear extracts. Ten of the twelve TIPs were found at roughly similar amounts in both proliferating and quiescent HeLa cells. However, a 96 kDa TIP appeared specifically in proliferating cells while a 231 kDa TIP was more abundant in quiescent cells. Growth state-selectivity of these TIPs may be indicative of their function in regulating topo II activity. Studies are currently in progress to clone and identify each TIP and determine whether their binding to topo II alters the enzyme's cleavage-religation equilibrium in the presence of topo II-targetting anticancer drugs.

R 308 CONCENTRATION-DEPENDENT DIFFERENCES IN THE MECHANISMS BY WHICH METHYL-XANTHINES POTENTIATE ETOPOSIDE CYTOTOXICITY IN HUMAN TUMOR CELLS, Richard B. Lock, Lori J. Rhodes and Olga V. Galperina, Departments of Medicine and Biochemistry, The J. Graham Brown Cancer Center, University of Louisville, Louisville, KY 40292

Exposure of human cervical carcinoma (HeLa) cells to caffeine (1-10 mM) following a 1 h treatment with the topoisomerase II inhibitor, etoposide, results in a 2- to 3-fold potentiation of cytotoxicity compared to etoposide alone. At a concentration of 1 mM, caffeine caused circumvention of etoposide-induced G2 arrest, and an increase in mitotic catastrophes resulting in giant cells containing fragmented nuclei. This is the generally accepted mechanism by which methylxanthines potentiate the cytotoxicity of DNA damaging agents in mammalian cell lines. However, at higher caffeine concentrations (5 and 10 mM) the number of mitotic catastrophes actually decreased, which did not correlate with the observation that the cytotoxicity of etoposide was increased. This appeared to occur for two reasons: 1) high concentrations of caffeine inhibited DNA synthesis and did not allow G1 cells to reach G2; and 2) the proportion of cells which underwent cell death via apoptosis significantly increased. Similar effects were observed with pentoxifylline, an agent with more potential for clinical use. Thus, methylxanthines can potentiate the cytotoxicity of anticancer agents in human tumor cells via two distinct mechanisms, identifying possibilities for specific therapeutic modulation. Using Chinese hamster ovary cells, potentiation of etoposide cytotoxicity was not observed at 5 and 10 mM caffeine concentrations, and apoptosis was not detected. We hypothesize that overexpression of *c-myc* in HeLa cells plays a role in the ability of caffeine to induce apoptosis following cell cycle perturbations caused by etoposide.

R 309 EXPRESSION OF BCL-2 IS HETEROGENEOUS IN ACUTE MYELOGENOUS LEUKEMIA (AML) AND MYELODYSPLASIA (MDS), Lynn C. Moscinski and Bobbye Hill, Department of Pathology, H. Lee Moffitt Cancer Center, University of S Florida, Tampa FL 33612

Bcl-2 functions as an inhibitor of apoptosis and prolongs cell survival. Normal bcl-2 transcripts have been detected in myeloid progenitor cells and are developmentally regulated, with high protein levels found in CD34+ progenitors. High levels of bcl-2 correlate with both the degree of differentiation in AML, as well as response to therapy. Since many anticancer agents function through initiation of programmed cell death, and bcl-2 levels are positively associated with resistance to apoptosis, it is hypothesized that more chemo-resistant hematopoietic neoplasms would express high levels of bcl-2. In order to test this proposal, we examined cells of 8 patients with MDS, 6 with AML arising in a background of MDS, and 10 with de novo AML. Cytospins were stained for bcl-2 using a sensitive immunohistochemical technique. The majority of blasts expressed bcl-2, although the level was heterogeneous within each patient, and higher in blasts from de novo AML. In cases of MDS, mature myeloid cells showed consistently higher levels than blasts, but always expressed less bcl-2 than admixed normal lymphocytes and plasma cells. In order to explain the difference in bcl-2 expression between de novo AML and MDS, proliferative fractions were compared. Samples from MDS patients contained a greater proportion of cells in S-phase. To test whether this finding was related to co-expression of P53, all samples were studied with an antibody which detects both the mutant and wild types. The majority of cases contained no detectable P53, and there was no correlation with bcl-2 or proliferative fraction. Thus, we demonstrate immunologically detectable bcl-2 in the majority of AML, with the highest levels in patients without a co-existent MDS. Bcl-2 is inversely correlated with proliferative fraction, suggesting a role in maintaining viability in low proliferative fraction hematologic malignancies. While P53 protein has been implicated as an antagonist of bcl-2, the low levels of bcl-2 in patients with MDS do not appear to be secondary to over-expression of P53.

R 310 CHARACTERIZATION OF TWO TUMOR MEMBRANE PROTEINS: INVOLVEMENT IN RECOGNITION AND EFFECTOR PHASES OF LYMPHOCYTE MEDIATED CYTOTOXICITY, Allen J. Norin, Ballabh Das, Mary Mondragon, Minoo Sadeghian, Shi-Zhen Tao, Departments of Medicine, Surgery and Anatomy & Cell Biology, SUNY Health Science Center, Brooklyn NY 11203

Lymphocytes that directly destroy tumor cells on contact (cytolytic lymphocytes) play an important protective role against cancer. Current concepts view cytolysis as a multi-step physiologic process involving: adhesion of effector and target cells, specific recognition, programming for lysis, and cell death. These reactions are likely controlled by receptor-ligand interactions that result in transmembrane signalling in both directions. We identified approximately 20 tumor plasma membrane proteins that bind to lymphocytes and therefore may be involved in the cytolytic process. Two of these molecules from the K562 cell line (NK susceptible) were purified by preparative PAGE for characterization. One of these membrane proteins, p38.5, bound specifically to a 72 KD receptor on NK cells but did not bind to T cells. Affinity purified antibody to p38.5 reacted strongly with NK susceptible tumors but only weakly or not at all with NK resistant tumors. Soluble p38.5 blocked NK cytotoxicity when lymphocytes were incubated with the protein pior to the addition of target cells. Structural and immunologic studies of another membrane protein, p51.5, demonstrated that it was similar or identical to the β subunit of H^+ transporting ATP synthase (β H^+ATPase) a known mitochrondrial protein. Antibody to the ATP binding domain of β H^+ATPase (anti-β) reacted with purified p51.5 that specifically bound to lymphocytes. Anti-β strongly inhibited (75% - 100%) LAK and NK cytotoxicity when lymphocytes were incubated with antibody pior to additional of target cells. These results indicate that (1) p38.5 may be involved in the recognition phase of NK cell mediated cytotoxicity (based on preferential binding to NK cells and strong expression limited to NK cell susceptible tumors) and (2) that p51.5 may be utilized in the effector phase/programming for lysis phase of the cytolytic process in that it functions in a variety of cells, ie naive NK cells, T cell-LAK and NK-LAK.

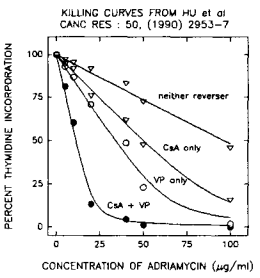
R 311 SERIAL ASSESSMENT OF MDR1 ACTIVITY IN ACUTE MYELOID LEUKEMIA (AML) SHOWS IN VIVO SELECTION OF CHEMORESISTANT POPULATIONS AND BOTH MDR1-MEDIATED AND NON MDR1-MEDIATED DRUG RESISTANCE, Deborah Rund, Department of Hematology, Hadassah University Hospital, Ein Kerem, Jerusalem, Israel 91120

14 patients with acute myeloid leukemia (AML) were studied prior to and after 1-4 cycles of chemotherapy using a functional analysis of MDR1 activity which uses rhodamine 123 staining and fluorescent activated cell sorter analysis. 8 patients were studied at presentation and 1-3 times post-chemotherapy, 3 at presentation and at relapse, and 3 at relapse and post-chemotherapy. 17 others were studied only once, either at presentation or after treatment. Overall, 15/31 (48%) of patients showed a rhodamine-dull population which increased in fluorescence with addition of an MDR1 inhibitor (verapamil), indicative of MDR1 gene activity. Of 10 patients dying of resistant leukemia, 5 (50%) showed no MDR1 activity using this analysis. The other 5 (50%) had significant MDR1 activity. In 3 patients with resistant AML, serial examinations showed a rhodamine-dull population which disappeared after therapy with a non-MDR1 mediated drug (high dose Ara C) In 2 others, MDR1 activity was never detected on repeated determinations. In 5 other patients with demonstrable MDR1 activity, serial studies showed progressive selection of a rhodamine-dull population after chemotherapy. These results demonstrate **A**: positive selection by chemotherapy on drug-resistant leukemic blasts and **B**: a significant prevalence of nonMDR1-mediated resistance to chemotherapy in AML.

R 312 MOLECULAR MECHANISM FOR SYNERGY BETWEEN REVERSERS OF THE MDR PUMP

Wilfred D. Stein, Dept of Biochemistry, Silberman Institute of Life Sciences, Hebrew University, JERUSALEM, ISRAEL, 91904

Cyclosporin A and verapamil reverse the MDR pump in that they decrease the LD50 for killing of drug-resistant cells by cytotoxins (Fig.). That they act synergistically to bring about reversal has been suggested and denied. An analysis of available data, on the basis of simple transport kinetics (the MDR pump pumping out drug in the face of a diffusive leak of the cytotoxin) shows that the effect of a combination of the two drugs (lowest data set) can be accounted for quantitatively (the line is computed) on the assumption that each reverser blocks outward pumping of the complementary reverser as well as of the cytotoxin, the mutual effect of the reversers leading to the observed synergism. The theory explains the finding that the dependence of cell killing on the concentration of cytoxin is not as given by simple Michaelis-Menten theory but by a higher dependence on drug concentration, since drug molecules block efflux of their partners, raising their internal concentration and hence effectiveness in cell killing.



R 313 MECHANISTIC STUDIES OF THE INHIBITION OF PROTEIN SYNTHESIS BY ANTITUMOR AGENTS, Peter L. Toogood and Bhagyashri V. SirDeshpande, Department of Chemistry, University of Michigan, Ann Arbor, MI 48109

One established mechanism of antineoplasia is inhibition of protein biosynthesis. However, while the selective inhibition of protein biosynthesis in prokaryotic cells has led to the development of many clinically effective antibacterials, the selective inhibition of protein biosynthesis in eukaryotic cells has not yet been sucessfully translated into an effective treatment for cancer. The potent antineoplastic natural product didemnin B is currently the subject of Phase II clinical studies in humans. Although the precise mechanism of action of this compound *in vivo* is currently unknown, whole cell data suggest that it may function as an inhibitor of protein biosynthesis. To test this hypothesis the inhibition of protein synthesis by didemnin B has been studied using *in vitro* biochemical assays. It has been shown that didemnin B (5 μM) will inhibit protein synthesis by eukaryotic cell lysates primed with tobacco mosaic virus RNA. This inhibition is demonstrated to occur at the stage of polypeptide elongation. A mechanism for this process is presented and experiments are described that give further details of the specific molecular interactions involved.

R 314 EXAMINATION OF DRUG SENSITIVITY IN HEAT RESISTANT HEPATOMA VARIANTS, Anikó Venetianer, Melinda Pirity and Anna Hevér-Szabó, Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

We have isolated glucocorticoid resistant, dedifferentiated rat hepatoma variants (for example clone 2), which exhibited deficient stress activation of the hsp68. Stable **heat resistant** mass populations and clonal variants of clone 2 cells were also isolated, some of which showed increased **drug resistance** to several anticancer drugs. The hsp68 inducibility was partially or fully restored in the heat resistant variants, the constitutive levels of certain major hsp-s were also elevated. Using increasing concentrations of anticancer drugs we isolated cell lines with higher drug resistance from clone 2 and from the heat resistant variants. These cells showed cross-resistance to other drugs and remained resistant to glucocorticoids. Although the heat resistant derivatives of clone 2 developed a moderate resistance to several drugs which was further increased by selection with increasing concentrations of colchicine, the degree of multidrug resistance was higher in the colchicine resistant clone 2 cells than in the colchicine and heat resistant variants. The induction deficiency of hsp68 was maintained in the cochicine resistant clone 2 cells and no increase in the constitutive level of major hsp-s was seen. Elevated MDR mRNA level was detectable in certain drug resistant variants. Our hepatoma variants may provide a valuable tool for examination of the mode of action of cell killing by drugs and heat and also the mechanisms responsible for the resistance to them.

R 315 **INVOLVEMENT OF GROWTH FACTOR SIGNALING PATHWAY IN MULTIDRUG RESISTANCE IN HUMAN BREAST CANCER CELLS.** K. Wosikowski, M. Saceda, M.B. Martin and S.E. Bates. Medicine Branch, National Cancer Institute, Bethesda, MD 20892 and Lombardi Cancer Research Center, Georgetown University, Washington, DC 20007.

Previous studies have demonstrated elevated levels of Epidermal Growth Factor (EGF) receptor in multidrug resistant (MDR) breast cancer cells. In this study we ask whether increased EGF receptor levels result in altered signal transduction that is associated with the development of P-glycoprotein mediated multidrug resistance. We utilized five sensitive parental human breast cancer cell lines and 20 resistant sublines. In the resistant sublines, either EGF, Transforming Growth Factor-α, amphiregulin or EGF receptor mRNA was overexpressed when compared to parental cells. Major changes in expression were not observed for c-*erb*B2, c-*erb*B3, Insulin Like Growth Factor-I, c-*myc* and c-*fos* expression in the MDR cells. In all the examined MDR sublines higher levels of phosphorylated tyrosine residues were observed, indicating an increased tyrosine kinase activity in these cells. After EGF stimulation tyrosine phosphorylation was increased confirming functionality of the EGF receptor. These data indicate that growth factors and their linked signal transduction pathways are upregulated after selection. Preliminary experiments suggest increased Raf kinase activation as well. Inactivation of the EGF receptor with an inhibiting antibody did not revert drug resistance or reduce the cellular tyrosine phosphorylation, suggesting that other intracellular components are involved. Studies are underway to define the mechanisms by which increased signaling pathway activation could contribute to the multidrug resistant phenotype.

Gene-Based Therapies; Clinical Trials of Novel Cancer Therapies

R 400 INHIBITION BY ALKYL-LYSOPHOSPHOLIPID OF TUMOR-INDUCED ANGIOGENESIS, SIGNAL TRANSDUCTION AND ADHESION MOLECULE EXPRESSION USING A HUMAN IMMORTALIZED MICROVASCULAR ENDOTHELIAL CELL LINE, Edwin W. Ades[1], Diane C. Bosse[1], W. Ralph Vogler[2], and Francisco J. Candal[1], [1]Centers for Disease Control and Prevention, National Center for Infectious Diseases, Scientific Resources Program, Biological Products Branch, Atlanta, GA 30333 and [2]Emory University, School of Medicine, Department of Hematology, Atlanta, GA 30322

Alkyl-lysophospholipids are a group of anti-cancer compounds that previously have been shown to have the unique feature of being selectively toxic to neoplastic tissues. One of these compounds, ET-18-OCH_3, has been used for purging bone marrow of cancer cells and in Phase I clinical trials. Tumor-induced angiogenesis has been directly correlated with tumor growth and metastasis. In this study, we examined the effect ET-18-OCH_3 has on a human microvascular endothelial cell line (HMEC-1) including the following functions: angiogenesis, cell adhesion molecule expression and cell junction integrity. We found that ET-18-OCH_3 (in vitro) reversibly inhibited induced angiogenesis at levels that did not affect viability. At lower concentrations, ET-18-OCH_3 down regulated cell adhesion molecule expression and affected cell to cell junction integrity. This data demonstrates this already versatile family of compounds to have additional targets of action.

R 401 PHENYLACETATE AND PHENYLBUTYRATE MODULATE THE EXPRESSION OF CELLULAR AND VIRAL GENES IN BURKITT'S LYMPHOMA. Matia Bar-Ner, Ian T. Magrath and Dvorit Samid. Pediatric Oncology Branch and Clinical Pharmacology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.

Phenylacetate (PA) and phenylbutyrate (PB) are differentiation and hypomethylation agents that can be administered to high serum levels with no significant toxicity. Both agents are currently being tested in clinical trials for cancer therapy. We have looked at various effects of these drugs on Burkitt's lymphoma cell lines in culture. While both drugs exerted a cytostatic effect, PB was found to be over three times more potent than PA on a molar basis. Sensitivity to the drugs greatly differed among the 12 cell lines tested, yet no correlation with Epstein-Barr virus (EBV) was observed. Changes in cell-cell and cell-substrate interactions were suggested by pronounced morphological changes (e.g. increased clumping or process formation). Moreover, Northern blot analysis demonstrated a modulated expression of genes that are implicated in growth control and immunogenicity (e.g. c-*myc* and HLA class I).

In Burkitt's lymphomas that are latently infected with EBV virus, viral antigens such as Latent Membrane Protein (LMP) may serve as a potential therapeutic target for the host immune system. LMP is not generally expressed in Burkitt's lymphoma in *vivo*, but our preliminary data suggest that this antigen can be upregulated by PB in Burkitt's lymphoma cell lines. We are further investigating the effects of PB on EBV gene expression, which may provide a mechanism for enhanced immunogenicity as well as for tumor cell lysis.

R 402 ALLO- AND AUTO- T-CELL RECOGNITION OF CML CELLS DOES NOT INVOLVE BCR/ABL FUSION PROTEINS. John Barrett, Antonio Guimaraes. Marrow Transplant Unit, NHLBI, National Institutes of Health, Bethesda, MD 20892.

It has been speculated that peptides derived from the junction region of the p210 bcr/abl fusion protein transcribed from the t9;22 translocation in chronic myelogenous leukemia (CML) are presented by MHC molecules for T-cell scrutiny. Such fusion peptides could function as leukemia-specific antigens. We monitored helper-T and cytotoxic-T cell precursor frequencies (HTLPf and CTLPf) to CML cells or bcr/abl peptides in normal individuals, in CML patients before and after marrow transplantation (BMT), using a limiting dilution assay. Stimulators were either T and B cell depleted CML PBMC, or autologous antigen presenting cells (APC) exposed to synthetic 9 or 18mer peptides representing the junction region of p210 b2a2 and b3a2. In 5 normal individuals there was a low precursor frequency to autologous APC exposed to both b2a2 and b3a2 18mer peptides (range 1/300,000-1/125,000). Five patients with CML had a high autologous HTLP frequency (range 1/12,000-1/45,000) which was unchanged by prior exposure of CML APC to bcr/abl peptides. Following BMT there was an increase in CTLPf to CML cells but not in HTLPf either to CML or to donor (autologous APC) exposed to bcr/abl peptides. These results do not exclude a role of bcr/abl fusion peptides in the immune response to CML, but suggest that other as yet undetermined antigens are of greater importance.

R 403 EXPRESSION OF THE CATALYTIC SUBUNIT (β) OF MITOCHONDRIAL ATP SYNTHASE ON THE PLASMA MEMBRANE OF TUMOR CELLS. Ballabh Das, Mary O. Mondragon, and Allen J. Norin. Departments of Medicine, Surgery and Anatomy & Cell Biology, SUNY Health Science Center, Brooklyn NY 11203

H^+transporting ATP synthase catalyzes the synthesis of ATP for cellular activities and is localized on the inner membranes of mitochondria. The β subunit (β H^+ATPase) is synthesized in the cytoplasm and translocated to mitochondria for assembly with other subunits of the enzyme. It bears the adenine nucleotide binding domain as well as ATP hydrolyzing activity and therefore is considered the catalytic subunit. Neoplastic cells and regenerating tissues have decreased number of mitochondria with altered structural and functional properties (e.g., ATP synthase deficient in β subunit content). Using a highly specific antibody (anti-β) against β H^+ATPase (directed against a peptide in the ATP binding region, Y311 to A331), we studied the expression of this protein on the plasma membrane of a wide variety of tumor cells and normal human tissues. Both immunoprecipitation (IP) and immunofluorescent (IF) methods were employed. Plasma membrane proteins were prepared from cells that were first surface labelled with biotin and then reacted with anti-β. If the protein is expressed on the cell surface then the immunoprecipitate would contain biotinylated protein of 50 to 55KD (the known wt range of mammalian mitochrondrial β subunit of H^+-ATPase). All tumor cell lines studied expressed this protein on their surface as determined by IP (9 leukemia/lymphoma, 7 carcinoma, 1 melanoma, 1 mastocytoma). Normal fresh human large intestine, lung, and lymphocytes did not express β H^+ATPase. A trace amount of β H^+ATPase was detected with cells of human small intestine. Flow cytometry and fluorescent microscopy demonstrated similar results with tumor cells and lymphocytes (i.e., viable tumor cells were fluorescent upon reaction with anti-β, whereas normal cells were negative). These results strongly suggest that a protein with immunologic identity to β H^+ ATPase is expressed on the plasma membrane of tumor cells but not on normal cells. The presence of the catalytic subunit of ATP synthase may have important implications in cancer biology and might serve a general marker for tumor cells.

R 404 ROLE OF G_{i2} PROTEINS IN TUMOR AND METASTASIS FORMATION IN MURINE MELANOMA. Sylvie Hermouet*, Sadie Aznavoorian#, Patricia S. Steeg# & Allen M. Spiegel*. *Molecular Pathophysiology Branch, NIDDK, #Laboratory of Pathology, NCI, NIH, Bethesda, MD 20892.

Pertussis toxin (PTX)-sensitive G proteins are involved in chemotactic responses in neutrophils and macrophages, and seem to play a role in cell motility, and possibly in metastasis formation, in murine melanoma cells: expression of PTX-sensitive G_{i2} proteins is increased in several highly metastatic clones of murine melanoma cell lines. Using a low metastatic potential clone (CL19) of the murine melanoma cell line K-1735, we obtained stable cell lines expressing wild-type and mutated forms of the α subunit of G_{i2}, and studied their cell growth and motility in vitro, and their ability to induce tumors and metastases in mice. Cell motility - either spontaneous, or type IV collagen or fibronectin-stimulated - was increased in all α_{i2}-transfected cells. However, overexpression of wild-type or mutated forms of $G\alpha_{i2}$ did not stimulate metastasis formation in animals. We observed opposite effects on cell growth, depending on the state of activation of G_{i2} proteins. Expression of constitutively activated $G\alpha_{i2}$ in K-1735 cells resulted in increased cell proliferation in vitro. Expression of a dominant inhibitory mutant of $G\alpha_{i2}$ strongly inhibited cell growth, as previously described in fibroblasts. Effects of the mutated forms of $G\alpha_{i2}$ on tumor formation in animals were also studied. We conclude that G_{i2}'s role in cell growth regulation is not limited to fibroblasts, and suggest that inactivation of G_{i2} is a new and efficient way to inhibit melanoma cell proliferation.

R 405 *Neu*-ONCOGENE-TARGETING CANCER THERAPY-- AN OVARIAN CANCER MODEL, Mien-Chie Hung, Leaf Huang, and Dihua Yu, Department of Tumor Biology, U.T.M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030 and Department of Pharmacology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261

Amplification or overexpression of *HER-2/neu* proto-oncogene occur frequently in many different types of human cancers and have been shown to correlate with decreased survival in breast and ovarian cancer patients. We have previously found that the ovarian carcinoma cell line SK-OV-3 overexpresses *HER-2/neu* mRNA. To further study the biological effect of *HER-2/neu* overexpression in SK-OV-3 cells, we injected i.p. such cells into female nu/nu mice and found that this cell line forms extensive abdominal tumors and ascites. From the ascites in an injected mouse, we established the SKOV3.ip1 cell line. Compared to the parental SK-OV-3 cells, the SKOV3.ip1 cell line expressed three-fold more *HER-2/neu*-encoded p185 proteins, showed higher cell-growth and DNA-synthesis rates, formed more colonies in soft agar, produced larger s.c. tumors and resulted in shorter survival of nu/nu mice after i.p. injection. These data indicate that the level of *HER-2/neu* overexpression may correlate with the degree of malignancy in these ovarian carcinoma cells. Since we had previously shown that the adenovirus 5 E1A gene product can suppress transformation and metastatic properties induced by mutation-activated rat *neu* oncogene in mouse embryo fibroblast cells, we further examined whether E1A can abrogate malignancy in *HER-2/neu*-overexpressing human ovarian cancer cells. We introduced the E1A gene into HER-2/*neu*-overexpressing SKOV3.ip1 cells and found that the E1A-expressing ovarian cancer cell lines had decreased *HER-2/neu*-encoded p185 expression and reduced malignancy, including a decreased ability to induce tumors in nu/nu mice. Therefore, we concluded that E1A is a tumor suppressor gene for HER-2/*neu*-overexpressing human ovarian cancer cells and may be useful in developing therapeutic reagents for these human cancers. To further examine whether E1A can function as a tumor suppressor gene for *neu*-overexpressing human cancer cells in living hosts, we used liposome-mediated direct gene transfer techniques to deliver E1A gene into the *neu*-overexpressing SKOV3 human ovarian cancer cells that have been growing in the peritoneol cavity of nude mice. Our preliminary results indicate that liposome-mediated E1A gene transfer inhibited *neu*-overexpressing human ovarian cancer cell growth and dissemination, and have led to prolonged survival of nude mice bearing *neu*-overexpressing SKOV3 cells.

R 406 PRE-INVESTIGATION ON PEGYLATION OF B3(Fv)PE38 FUSION TOXIN BY CYSTEINE MUTAGENESIS OF *PSEUDOMONAS* EXOTOXIN A SURFACE EXPOSED RESIDUES MODIFIED WITH PEG IN A SITE-SPECIFIC MANNER, Chien-Tsun Kuan, Qing-cheng Wang and Ira Pastan, Laboratory of Molecular Biology, DCBDC, NCI, NIH, Bethesda, MD 20892

The single-chain recombinant toxin B3(Fv)PE38 is specifically toxic to cells containing the B3 antigen and causes complete regression of human cancer xenografts in nude mice. To facilitate therapeutic applications, such chimeric toxins have two features that need to be improved. One is the short lifetime of these proteins in the circulation and the other is their immunogenicity. Modification of proteins with polyethylene glycol (PEG) has been shown to prolong the circulating plasma lifetime and may reduce or eliminate immunogenicity. We have used PE as a model to define whether modification of the translocation domain of the toxin with PEG will maintain its cytotoxicity. To investigate the effect of PEGylation on specific residues located on the surface of domain II of PE, we substituted cysteine, for each of the five most exposed surface amino acids (H276, E282, N306, R313, and E327) in domain II. These cysteines can serve as unique sites for PEG modification. When the PEcys proteins were conjugated with ovalbumin using a cleavable disulfide linkage, cytotoxicity was retained, but it was lost with a non-cleavable thioether linkage. In contrast, cytotoxicity was maintained when PEcys mutants were coupled to mPEG, using either a disulfide or a thioether linkage. Unexpectedly in some cases, the thioether conjugate was more active than the disulfide linkage. Pharmacokinetic studies on one of the PEGylated molecules (R313C) showed that the mean residence time (t 1/2) was prolonged to 72 min, compared to 20 min for unPEGylated PEcys(R313C). These studies show it is possible to derivatize PE at specific residues in domain II, maintain significant cytotoxic activity and alter pharmacokinetics. These studies also indicate that large mPEG molecules can be translocated to the cytosol while still attached to domain II of PE. PEG modification of B3(Fv)PE38 cysteine mutants is being investigated.

R 407 POOR MIGRATION OF CLONED HUMAN T CELLS GRAFTED IN SCID MICE, V Malkovska, R Abonour, K Schell and F Cigel, Department of Medicine, University of Wisconsin, Madison, WI 53792

The incubation of human peripheral blood lymphocytes (PBL) with irradiated Daudi lymphoma cells induces selective polyclonal proliferation of Vγ9/Vδ2 T-cells with high cytotoxic activity against Daudi. We reported previously that these cells generated *in vitro*, or induced in peritoneal cavity of mice with severe combined immune deficiency (SCID mice), prolonged survival of mice given a lethal dose of live Daudi lymphoma. In the same immunotherapy model, Daudi-specific cytotoxic Vγ9/Vδ2 T-cell clones had much lower antitumor activity. We therefore studied the survival and migration of cloned human T-cells after i.p. inoculation of SCID mice. Mice were conditioned with Cyclophosphamide (400mg/kg) and inoculated with $3x10^7$ cloned Vγ9/Vδ2 T-cells i.p. followed by daily 5000 IU IL-2 s.c. At 2-3 weeks 1-15% TCR-γδ-1 positive cells were detected by flow cytometry in the peritoneal cavity of 5/6 mice. In all animals <1% human T-cells were found in the blood, spleen, pooled lymph nodes and bone marrow. This lack of Vγ9/Vδ2 T-cell migration outside the peritoneal cavity was also observed in 2 animals bearing Daudi lymphoma. In 6/6 mice that received αβ T-cell clones in the same schedule, 3-45% human αβ T-cells were detected at 2-3 weeks in the peritoneal cavity, 0-5% in the spleen and <1% in the blood, pooled lymph nodes and bone marrow. In contrast, 5/6 mice that received fresh $3x10^7$ PBL, 7-20% human T-cells were detected at 2-3 weeks in the blood, 1-8% in the spleen, 12-31% in pooled lymph nodes and 0-1% in the bone marrow. These data suggest that cloned human T-cells can lose their ability to migrate effectively outside SCID peritoneal cavity. Thus the SCID mouse may not be a suitable model for testing adoptive immunotherapy with cloned human T-cells.

R 408 CANCER THERAPY WITH BETA-PARTICLE EMITTING IMMUNOCONJUGATES: TUMOR UPTAKE AND CELLULAR METABOLISM OF A NEURO-BLASTOMA-SPECIFIC, ^{67}CU-LABELLED ANTIBODY, I. Novak-Hofer, H. Amstutz*, K. Zimmermann, R. Schwarzbach, J.J. Morgenthaler* and P.A. Schubiger, Paul Scherrer Institute, CH-5232 Villigen and *Swiss Red Cross Laboratories, Berne, Switzerland.

Tumor specific antibody conjugates chelating beta-particle emitting radionuclides are expected to bring a therapeutic advantage over ^{131}I-labelled preparations currently used in radioimmunotherapy (RIT) trials. ^{67}Cu is considered a promising nuclide for RIT because of its suitable half life (2.6 days), its radiation characteristics (mean beta energy 141 keV) and because it forms complexes of high in vivo stability. We derivatized a neuroblastoma-specific chimeric antibody (chCE7) with a macrocyclic amine ligand (14N4) and labelled it with ^{67}Cu (produced by cyclotron irradiation of a Zn target and purified by ion exchange chromatography). ^{67}Cu-chCE7 had a specific activity of 0.3-0.5 μCi/μg and was fully immunoreactive. Tumor uptake of ^{67}Cu-chCE7 in nude mice bearing neuroblastoma xenografts, was consistently higher (33.7 ± 2.8 % I.D./g, 4 days post injection) than of ^{125}I-chCE7 (16.3 ± 1.0 % I.D./g, 4d p.i.). Tumor/blood ratios of ^{67}Cu-chCE7 increased from 4.4 on day 1 to 25.5 on day 7 p.i., compared with tumor/blood ratios of 2.6 to 3.4 achieved with ^{125}I-chCE7 over the same time period. Mab chCE7 is internalized into neuroblastoma cells and cellular degradation of ^{67}Cu-chCE7 and ^{125}I-chCE7 was investigated. It was found that the rate and the extent of internalization of both preparations is similar. Degradation in both cases was inhibited by chloroquine, indicating similar intracellular routing to endo- or lysosomal vesicles. The main terminal degradation product of ^{125}I-chCE7 is iodotyrosine, which is rapidly released from cells, leading to a loss of cell bound radioactivity over time. In contrast, the terminal degradation product of ^{67}Cu-chCE7 consists of the ^{67}Cu-14N4 complex which is retained within cells, leading to intracellular accumulation of radioactivity. This effect may provide an additional therapeutic benefit of ^{67}Cu-labelled immunoconjugates.

R 409 **NDP-KINASE AND LEUCINE AMINOPEPTIDASE: TWO PROGNOSTIC FACTORS OF CELLULAR INVASIVENESS AND METASTASIS OF HUMAN BRAIN TUMOURS.** **Gabriel Pulido-Cejudo [3], Keri Jamison [3], Herman Hugenholtz [2], B.Lach [1], M.Anne Smith [3] and José Campione-Piccardo [3].** **[1]Department of Pathology, Ottawa Civic Hospital. [2]Department of Neurosurgery, Ottawa General Hospital. [3]National Laboratory for Viral Oncology, LCDC Canada K1A OL2.**

The identification of cellular markers to define tumour metastatic behaviour is crucial in determining prognosis and selective therapy. In this study, serum levels of Leucine Aminopeptidase (LAP) and cellular NDP-kinase (NDP-K) activities determined in cell cultures of primary and secondary brain tumours (metastatic breast and lung carcinomas) were used to assess tumour malignancy. Based on both the net increase in seric LAP exopeptidase activity and tumour histopathology, three grades of tumour malignancy were tentatively defined:

TUMOUR GRADE	LAP ACTIVITY (U/ug)
1. Highly malignant/metastatic	1×10^{-5} to 2×10^{-4}
2. Malignant/metastatic	2×10^{-6} to 1×10^{-5}
3. Poorly malignant/metastatic	1×10^{-7} to 2×10^{-6}

Cytosolic and stromal NDP-K activities were determined in gemistocytic astrocytomas, glioblastoma multiforme, metatastic breast and lung carcinomas ranked as grades 1-2. Western blot analysis demonstrated a greater proportion of the enzyme in the stromal fractions than that found in the cytosol. In contrast, NDP-K activity staining after native PAGE as well as total enzyme activity showed that most of the enzyme activity was present in the cytosolic fraction with negligible enzyme activity in the stromal preparations. In addition, cytosolic NDP-K activity staining revealed three distinct species in comparison to a single cytosolic form observed in benign tumours. Hence, based on seric LAP levels as well as on NDP-K activity distribution and oligomeric composition of the cytosolic NDP-K a quantitative appraisement of tumour malignancy can be achieved. In conclusion, malignant and metastatic brain tumours possess a greater proportion of stromal inactive NDP-K in comparison to a lesser cytosolic but active NDP-K activity. The role of membrane bound and cytosolic NDP-Kinase activities in cellular proliferation and oncogenesis will be discussed.

R 410 RECEPTOR TARGETED BORON CONTAINING PEPTIDES FOR BORON NEUTRON CAPTURE THERAPY OF CANCER, Peggy A. Radel and Stephen B. Kahl, Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-0446

As part of our ongoing efforts in design and synthesis of selective ^{10}boron containing drugs for Boron Neutron Capture Therapy of cancer, we are developing an approach to utilize peptide analogs of known receptor binding ligands. The receptor systems we are examining are upregulated in cancers of interest, including glioma, melanoma, prostate, pituitary, and breast. The substantial increase of receptor numbers in these tumor cells may allow us to target drug delivery via these receptors to create the gradient of ^{10}boron between neoplastic cells and the adjacent normal cells necessary for effective therapy without normal tissue damage. Boron Neutron Capture Therapy has the potential to be a very precise, efficient, and non-invasive method to kill tumors. This binary therapy produces alpha particles only where both non toxic ^{10}boron-containing drug and non-ionizing neutrons converge and react. The cytotoxic effect is due to the damage caused by the resultant alpha particle as it loses its large kinetic energy within 10-14 microns, which is about one cell diameter.

A major hurdle to progress to clinical acceptance of this therapy is the lack of selective drugs that localize well to tumors and which provide the roughly 20-30 ppm ^{10}boron that is required for effective therapy. We believe that peptide analogs of receptor binding agonists or antagonists, carrying ^{10}boron in the form of the very stable C_2B_{10}-ortho-carborane cage, can import sufficient ^{10}boron into neoplastic cells. These cage compounds are incorporated into the drug as an unnatural amino acid, carboranylalanine. For this clinical application, peptidal drugs may be tolerated and their *in vivo* lifetimes extended by the presence of this unnatural amino acid. We will present the rationale for utilizing carboranylalanine as an isostere of phenylalanine in these analogs, and the enantioselective synthesis of both ^{10}boron enriched and natural isotope distribution amino acids (L and D). The design and synthesis of carboranylalanyl substituted peptide analogs will be discussed.

R 411 A NOVEL SERIES OF LOW MOLECULAR WEIGHT PHOSPHOLIPID SIGNALING INHIBITORS BLOCK TUMOR CELL ADHESION, 92KD METALLOPROTEASE EXPRESSION AND ANGIOGENESIS. JW Singer, P Klein, P Brown, R Chaney, S Bursten, and G Rice. Cell Therapeutics, Inc, Seattle WA 98119.

For tumors to develop, invade, and metastasize, they must vascularize, adhere to, and subsequently invade endothelial cell-lined vessels. Vascularity is induced by production by tumor cells of one or more endothelial cell growth factors including basic fibroblast growth factors (FGF), vascular endothelial growth factor (VEGF), and PDGF. Each of these cytokines induces multiple cellular events including calcium fluxing, cell migration, and mitogenesis, however the pathway leading to mitogenesis has not been previously identified. Abolition of phosphatidylinositol turnover by a point mutation in the FGF receptor suppressed calcium fluxing but not mitogenesis (Nature 358:678, 1992). Studies in our laboratory of lipid extracts of endothelial cells using HPLC separation followed by FAB mass spectroscopy demonstrate that within 5 seconds of application of FGF or PDGF, there is a rapid increase in phosphatidic acid (PA) and diacylglycerol (DAG) containing oleic, linoleic acid or myristate but not arachidonic acid in the sn2 position. Suppression of PA synthesis by low molecular weight specific inhibitors of the enzyme that converts lyso PA to PA (lysophosphatidic acid acyltransferase; LPAAT) suppresses mitogenesis but not PDGF or FGF induced calcium fluxing or smooth muscle cell migration. Stimulation of endothelial cells in the presence of these compounds inhibits the production of PAand causes accumulation of the PA precursor, lysoPA. The most potent of these compounds (CT-3532) suppresses human umbilical vein endothelial cell (HUVEC) and smooth muscle cell proliferation induced by FGF, VEGF, and PDGF in thymidine incorporation assays and also suppresses HUVEC matrigel invasion with a 50 percent inhibitory concentration (IC_{50}) of approximately 100 nM. CT-2532 is non-toxic to HUVEC's at concentrations of >50 μM. CT-3532 also may affect tumor invasion as it completely inhibits the IL-1 or TNF α induction of the 92kd metalloprotease in THP1 cells by gelatin gel and northern blot analysis at 10μM. The effect of this compound on the ability of tumor cells to adhere to activated TNFα or IL-1 activated HUVEC's was tested as a measure of the potential ability of this drug to suppress metastases. CT-3532 inhibited adhesion of the HT-29 colon cancer and the HT-177 large cell lung cancer cell lines to activated HUVEC's with an IC_{50} of approximately 1.0μM. FACS analysis of HUVEC's demonstrated that CT-3532 suppresses the cytokine induced upregulation of both VCAM and ICAM. Compounds such as CT-3532 are of potential interest as antitumor agents in vivo due to their activity on tumor induced angiogenesis, adhesion, and invasion.

R 412 IN VITRO AND IN VIVO ACTIVITIES OF A DOXORUBICIN PRODRUG IN COMBINATION WITH A MONOCLONAL ANTIBODY-β-LACTAMASE CONJUGATE, Håkan P. Svensson, Peter D. Senter, Vivekananda M. Vrudhula, and Philip M. Wallace. Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121

Cephalosporin doxorubicin (C-Dox) was designed as an anticancer prodrug that could be activated in a site-specific manner by monoclonal antibody-β-lactamase conjugates targeted to antigens present on tumor cell surfaces. Purified β-lactamases from E. coli, B. cereus, and E. cloacae catalyzed the release of Dox from C-Dox through a fragmentation reaction which occurs after the β-lactam ring of C-Dox is hydrolyzed. The E. cloacae β-lactamase (ECIβL) was chemically attached to either whole, $F(ab')_2$, or F(ab') fragments of the L6 monoclonal antibody forming conjugates that could bind to antigens on the H2981 human lung adenocarcinoma cell line. Cytotoxicity experiments demonstrated that C-Dox was significantly less cytotoxic than Dox on H2981 cells. Treatment of the cells with L6-ECIβL followed by C-Dox resulted in a level of cytotoxic activity that was comparable to Dox. In vivo experiments were performed using mice that had staged H2981 s.c. tumor xenografts. The antitumor activity of the L6-ECIβL/C-Dox combination was significantly greater than that of C-Dox or Dox alone, as well as that of a non-binding control conjugate/C-Dox combination. A rationale for this enhanced activity will be presented.

R 413 THE OVARIAN CARCINOMA MARKER FOLATE RECEPTOR CONTRIBUTES TO THE IN VITRO AND IN VIVO CELL GROWTH: EVIDENCES BY TRANSFECTION OF THE cDNA INTO NIH/3T3 CELLS, Tomassetti A., F. Bottero, S. Canevari, S. Miotti, S. Mènard and M. I. Colnaghi, Experimental Oncology E, Istituto Nazionale per lo Studio e la Cura dei Tumori, Via Venazian 1, Milan, Italy

Folic acid and its reduced compound are essential vitamins for cell growth. The uptake of these vitamins into the cells involves an high affinity folate receptor (FR) and a membrane carrier. The first, named folate binding protein, binds oxidized folate and analogues with an affinity below 1 nM, and allows the internalization of folates through the formation of a receptor-vitamin complex that dissociated by acidification. Thus, while the vitamin is released inside the cell, the FR recycles on the membrane surface. The FR is homogeneously overexpressed on the membrane of 90% of ovarian carcinomas. A soluble form of the FR was detected in ascitic fluids and in the sera from ovarian carcinoma patients. As the FR is a potential marker for ovarian carcinoma, a model has been developed to investigate the biochemical and biological properties of this molecule. NIH/3T3 cells, which do not endogenously express FR, were transfected with a vector containing the cDNA for the FR cloned from the ovarian carcinoma cell line IGROV1. The FR expressed showed features identical to those of the protein produced by IGROV1 cells. The FR is expressed on the cell membrane as a glycosylphosphatidyl-inositol-linked protein and is shed into the culture medium of the NIH/3T3 transfectants. The FR from transfected NIH/3T3 cells bound folic acid and the cells internalized about 30-fold more folic acid than mock-transfected cells. A growth analysis of transfected NIH/3T3 cells was performed at physiological folate concentration (about 20 nM) and also at concentration lower than 1 nM. As IGROV1 cells, transfected NIH/3T3 maintained growth rates independent of folate concentration in the medium. To test the effect of FR on proliferation in vivo, FR- and mock-transfected NIH/3T3 were subcutaneously transplanted into nude mice. Tumors originated from mock-transfected cells grew more slowly. The difference in proliferation capacity between the two cell types was more striking when tumors weights were compared: tumors from FR-transfected NIH/3T3 weighed 3 times more than those from the mock-transfected cells. Based on our results, we hypothesized that FR expression contributes to the in vivo growth of ovarian carcinoma.

R 414 C-*erb*B-2/*neu* OVEREXPRESSION ENHANCED METASTATIC POTENTIAL IN HUMAN LUNG CANCER CELLS BY INDUCTION OF METASTASIS-ASSOCIATED PROPERTIES, Dihua Yu, Shan-Shue Wang, Kim M. Dulski, Garth L. Nicolson, and Mien-Chie Hung, Department of Tumor Biology, U.T.M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030

We and others have previously reported high levels of expression of the c-*erb*B-2/*neu* gene in non small cell lung cancer (NSCLC) cell lines and primary tumors. We have also found that $p185^{neu}$ expression was correlated with lymph node metastasis in the squamous cell carcinomas. To systematically investigate the potential role of c-*erb*B-2/*neu* gene in lung cancer metastasis, we introduced the human c-*erb*B-2/*neu* gene into the very low $p185^{neu}$ expressing NCI-H460 human NSCLC cells. We then examined the metastatic potentials among the parental NCI-H460 cells and the NCI-H460 stable transfectants that expressed increased levels of $p185^{neu}$. Compared to the parental NCI-H460 cells, the $p185^{neu}$ overexpressing NCI-H460 transfectants produced significantly more pulmonary and extrapulmonary metastatic tumors in nude mice upon tail vein injection into nude mice. Enhanced tumor metastatic potential *in vivo* was accompanied by increase of invasiveness *in vitro*. In addition, important steps in the invasion and metastasis process, such as secretion of basement membrane-degradative enzymes and migration through layer of reconstituted basement membrane (Matrigel), were also enhanced in the $p185^{neu}$ overexpressing NCI-H460 transfectants. Moreover, scanning electron microscopy revealed that the $p185^{neu}$ overexpressing NCI-H460 transfectants had significantly more microvilli and membrane protrusions than the parental cells which provided a clear structural feature for increased cell motility. The results demonstrate that $p185^{neu}$ can enhance metastatic potential of NCI-H460 human lung cancer cells. The mechanism by which $p185^{neu}$ induces higher metastatic potential in human NSCLC is to promote invasion steps of the metastatic cascade. These findings support the notion that c-*erb*B-2/*neu* oncogene play a criticle role in certain human malignancies including NSCLC. Therefore, more aggressive therapy might be beneficial to those NSCLC patients whose tumors express high levels of $p185^{neu}$ and hence may have higher risk for metastasis.

R 500 GENE THERAPY OF CANCER USING CYTOKINE EXPRESSING RECOMBINANT ADENOVIRUS VECTORS
Christina Addison[+], Robert Ralston, Jack Gauldie[*], Frank L. Graham[+*] Departments of Biology[+] and Pathology[*], McMaster University, Hamilton Ontario, Canada; Department of Molecular Virology, Chiron Corporation, Emeryville California

The anti-tumour potential of a variety of cytokines has been demonstrated both in vitro and in vivo by a number of different laboratories. Recently, experiments involving the expression of interleukin-2 (IL-2) in tumour cells have been undertaken in order to study its therapeutic value in the treatment of cancer (Fearon et al; 1990; *Cell* **60**; 397). We are exploring the potential utility of human adenovirus vectors for delivery of cytokine genes to tumour cells.

As a gene delivery system adenovirus has several advantages over other commonly used gene therapy vectors including it's ability to infect non-replicating cells, it's tropism for a variety of human tissues and the ability to generate replication deficient virus by deletion of the E1 region.

We have inserted the human IL-2 cDNA under the control of the human cytomegalovirus IE promoter into an adenovirus type 5 vector. This vector has been shown to direct the expression of a protein recognized by monoclonal antibodies against huIL-2 (as determined by ELISA and Western blot analysis). Quantitation of expression of IL-2 by AdIL-2 has shown that 2-8 ug per 10^6 infected human cells can be synthesized over a period of 48 hours, with expression of the gene persisting for at least 7 days post infection. Initial experiments using the mouse B16 tumour model for melanoma have shown a delay in tumour progression after in vitro infection of B16 cells with AdIL-2. The system appears very promising for cancer therapy and experiments are currently underway to optimize the anti-tumour potential of this vector.

R 501 GENE TRANSFER WITH ADENO-ASSOCIATED VIRAL PLASMID DNA:LIPOSOME COMPLEXES FOR HUMAN CANCER GENE THERAPY. Elisa Brunette, Deepa Murugesh, Lydia Kilinski, Maureen McNally, Jane Lebkowski, Thomas B. Okarma and Ramila Philip. Applied Immune Sciences, Inc., Santa Clara, CA 95054

Advances in gene therapy depend largely upon the development of delivery systems capable of efficiently introducing DNA into the target cells. In this study, cationic liposomes were used to facilitate adeno-associated viral plasmid transfections into primary and cultured cell types. AAV plasmid DNA complexed with liposomes efficiently transfected cultured cell lines and primary tumor cells. In addition to high level expression, this combination of AAV plasmid:liposomes induced long term (>30 days) expression of the introduced gene in contrast to the short term expression demonstrated with typical liposome mediated transfection using standard plasmids. Primary breast and ovarian tumor cells were transfectable using the AAV plasmid DNA:liposome complexes. Transfected primary and cultured tumor cells were able to express the transgene product even after lethal irradiation. Using the liposome:AAV plasmid complexes, we have also explored methods to increase the efficacy of tumor specific tumor infiltrating lymphocytes (TIL) by stimulating the T cells with gene modified autologous tumor cells *in vitro*. Phenotype, cytotoxicity and T cell receptor analyses have demonstrated that although TIL show specificity when they are freshly isolated from tumor, long term culture in rIL-2 induces polyclonal expansion and loss of tumor specificity. However, when the expanded T cells were stimulated with gene modified autologous tumor, enrichment of tumor specific T cells occurred. We are currently using the above described gene delivery system to insert cytokine cDNA into primary human breast and ovarian tumor cells for *in vitro* sensitization of T cells.

R 502 REDIRECTING THE CYTOTOXIC CELLS OF THE IMMUNE SYSTEM USING RETROVIRAL MEDIATED TRANSDUCTION OF CHIMERIC ANTIGEN RECEPTOR GENES. M. Finer, T. Dull, K. Cooke, L. Qin, D. Ruff, K. Zsebo and M. Roberts. Cell Genesys, Foster City, CA 94404

We have developed a strategy to redirect the specificity of the hematopoietic system's cytotoxic cells toward targets such as virally infected or tumor cells. MHC unrestricted chimeric antigen receptor molecules (UR), encoding a single chain antibody fused to the CD3 complex associated- z chain, have been efficiently introduced into either human CD8+ T cells (up to 40% transduction), murine hematopoietic stem cells and human CD34+ cells (up to 10% and 45% transduction, respectively) using the novel *kat* transient retroviral production system. The *kat* system enables delivery of UR constructs without the need to generate stable retroviral producer clones for each construct. *kat* vectors expressed UR constructs at levels equivalent to those observed in NIH 3T3 cells, sufficient to purify transduced cells to homogeneity by immunoaffinity chromatography. Introduction of universal receptors into CD8+ T cells *in vitro* results in the activation of a number of T cell effector functions including cytokine secretion, proliferation and cytolysis, in response to target cells expressing the appropriate target antigen. The ability to redirect the cytotoxicity of myeloid or NK cells derived from transduced murine stem cells *in vivo*, and granulocytes derived from transduced human CD34+ cells *in vitro* are being evaluated.

R 503 DETECTION OF BROMODEOXYURIDINE INCORPORATION BY ALTERATION OF THE FLUORESCENCE FROM NUCLEIC ACID BINDING DYES USING ONLY AN ARGON ION LASER, Tom Frey, Becton Dickinson Immunocytometry Systems, 2350 Qume Drive, San Jose, CA 95131

A method was developed that uses two DNA dyes to detect the incorporation of bromodeoxyuridine (BrdU) into cellular DNA and requires only the use of 488 nm excitation from an Argon ion laser. The fluorescence of each of the three dyes thiazole blue, TO-PRO-3, and LDS-751 was found to be altered by the presence of BrdU in DNA. Pairing any one of these dyes with propidium iodide (PI) for flow cytometry allowed the differentiation of cells containing BrdU from BrdU unlabeled cells. These dyes can be excited directly (LDS-751) or indirectly (TO-PRO-3 or thiazole blue) by 488 nm light from an argon ion laser. Indirect excitation of TO-PRO-3 or thiazole blue was provided by resonance energy transfer from PI. Other dyes, including thiazole orange, TO-PRO-1, rhodamine 800, oxazine 750, or 7-aminoactinomycin D, were found to be unaltered by the presence of BrdU in DNA. Experiments to address the mechanism of the fluorescence changes seen with TO-PRO-3 were carried out. The fluorescence from TO-PRO-3 was found to be enhanced in the presence of BrdU. This enhanced fluorescence of TO-PRO-3 appears to lead to an enhancement of the energy transfer to TO-PRO-3 from PI, in turn leading to a decrease in PI fluorescence. Results were also obtained showing that BrdU detection using PI and TO-PRO-3 is compatible with immunofluorescence staining with FITC-labeled antibodies, thus allowing simultaneous single laser based measurement of BrdU incorporation into specific, antibody-identified cell types.

R 504 Retroviral vector mediated γ-IFN gene transfer to tumor cells for cancer therapy. Ramarao Gangavalli[1], Tammy McCallister[1], Shankar Nayak[2], Robert Dillman[2], Joeseph Rosenblatt[3], Robert Seeger[4], Malcolm Brenner[5], Carole Coze[5], Kim Lyerly[6], Hilliard Siegler[6], Doug Jolly[1] and Jack Barber[1]. [1]Viagene, Inc., San Diego, CA 92121, [2]Hoag Memorial Hospital, Newport Beach, CA, [3]UCLA School of Medicine, L.A., CA, [4]Children's Hospital, USC School of Medicine, L. A., CA, [5]St. Jude Children's Hospital, Memphis, TN and [6]Department of Surgery, Duke University Medical Center, Durham, NC.

A retroviral-vector (Mo-MLV based) mediated, cytokine gene (γ-IFN) transfer system was used to transduce primary human tumor cells. Primary human Melanoma, Renal cell carcinoma, Neuroblastoma, Ovarian carcinoma and Breast carcinoma cells were successfully transduced with retroviral vectors expressing β-gal and γ-IFN. γ-IFN and β-gal vectors, at a multiplicity of infection (MOI) of 1-10 vector particles per cell, transduced tumor cells with variable transduction efficiency (10-100% - 10 melanomas tested). Transduction efficiency was optimized by varying 1) polycation concentration, 2) MOIs, 3) number of exposures with vector, 4) G418 concentrations and 5) exposure time in selection of transduced cells. γ-IFN transduced melanoma and Neuroblastoma cells secreted varying amounts of biologically active γ-IFN for 20-40 days. Melanoma and Neuroblastoma cells that expressed γ-IFN exhibited increased surface expression of HLA class I antigens and were more active in *in vitro* stimulating cytolytic activity of lymphocytes from tumor bearing patients against their own tumor target cells. Several Neuroblastoma cell lines showed increased expression of both MHC Class I and II antigens. We have begun a clinical trial of human γ-IFN transduced autologous melanoma cells and plan to use γ-IFN transduced autologous tumor cells to boost host immune responses as a potential therapy for other tumor types as well.

R 505 STIMULATION OF ANTI-TUMOR IMMUNITY BY TGFβ:GENETIC MODIFICATION OF MURINE TUMOR TO EXPRESS TGFβ INDUCES TUMOR REGRESSION, Stephen E. Karp, Helen L. Chan & Michael P. Hier, Departments of Surgery and Oncology, Jewish General Hospital/McGill University, Montreal, Canada H3T 1E2

TGFβ is a pleiotropic cytokine expressed in many different tumors. It is generally held that TGFβ has suppressive activity on the anti-tumor immune response. In order to examine the consequences of secretion of TGFβ on the immune response to a growing tumor, a weakly immunogenic murine fibrosarcoma, WP4, was genetically altered to express TGFβ. The cDNA encoding for murine TGFβ1 was cloned into the eukaryotic expression vector pRep7. pRep7-TGFβ was inserted into WP4 by calcium/phosphate transfection. Hygromycin resistant clones were screened by bioassay for TGFβ expression. Selected clones (TGF1, TGF11, & TGF37) secreted greater than 5000 pg/5 x 10^5 cells/24 hours. Non transfected WP4 NV cells and hygromycin only resistant clones did secrete TGFβ, however secretion was markedly lower, less than 300 pg. Expression of TGFβ had no effect on cell morphology or proliferation. In order to evaluate the effects of expression of TGFβ on tumor growth, control or TGFβ secreting WP4 was injected into syngeneic mice. To our surprise, growth of TGFβ secreting clones was markedly inhibited in vivo. A clone that secreted less TGFβ, TGF-8, grew to a size intermediate to clones 1, 11, and 37. The difference in tumor sizes between NV or the hygromycin resistant clone, and TGF-1, TGF-11, or TGF-37 was statistically significant with a $p2< 0.046$ (Kruskall Wallis test). Regression was immunologically mediated as sublethal irradiation of the mice prior to tumor injection abrogated tumor regression. This unexpected finding questions the assumption that TGFβ is suppressive in vivo to the anti-tumor immune response. We are presently investigating the mechanisms of immune stimulation in this tumor system.

R 506 RETROVIRAL GENE TRANSFER OF ANTI-RAS RIBOZYME INTO TUMOR CELLS WITH ACTIVATED RAS ONCOGENE, Mingxia Li, Robert Kramer, Department of Oncology and Immunology, Medical Research Division, American Cyanamid Company, Pearl River, NY 10965

Hammerhead ribozymes targeting the activated H-Ras (12 val) oncogene were designed with RNA folding computer software (MFold from GCG). Their efficiency and specificity in mediating the cleavage of the H-Ras transcript was analyzed in a cell-free system. The anti-Ras ribozyme cleaved the mutant but not wild type H-Ras substrate. Mutations in the catalytic center of the ribozyme abolished the activity of the ribozyme. The effect of modifications in the stem 3 of the ribozyme and the presence of other RNA sequences adjacent to the ribozyme in the same RNA transcript was also studied. For example, the ribozymes maintained their catalytic activity when ligated to a tRNA molecule in the same transcript. The anti-Ras ribozymes were cloned into Moloney murine leukemia virus based double copy retroviral vectors under the control of a human tRNA promoter. These retroviral vectors were constructed to carry selectable markers of Neo, mutant DHFR or PAC gene to enrich the transduced target cells. The expression of the ribozymes are being analyzed in cells with or without the activated H-Ras oncogene, and their efficiency in reducing Ras oncogene expression is being studied.

R 507 ADOPTIVE IMMUNOTHERAPY OF MALIGNANT DISEASES USING GENE-MODIFIED TUMOR-SPECIFIC CD8$^+$ CYTOTOXIC T LYMPHOCYTES.

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Adoptive immunotherapy using tumor-specific CD8$^+$ cytotoxic T lymphocytes (CTL) has been effective in experimental models, but has achieved only limited success in the clinic. We are investigating the possibility of improving the efficacy of tumor-specific CTL by retrovirus-mediated gene transfer. The engraftment of adoptively-transferred CTL *in vivo* is usually poor in the absence of either exogenous IL-2 or CD4$^+$ helper T lymphocytes. However, animal studies have demonstrated that helper-independent CTL are capable of proliferating *in vivo* in response to T cell activation and persisting *in vivo* for prolonged periods of time. Accordingly, we are developing retroviral expression vectors which alleviate the requirement for exogenous IL-2 or CD4$^+$ helper T lymphocytes, and convert conventional CTL into helper-independent CTL. The ability to generate and expand helper-independent tumor-specific CTL would greatly improve the clinical utility of adoptive immunotherapy for malignant diseases. Recognizing the need for improved T cell expression vectors, we devised protocols for the efficient transient transfection of primary murine and human T cells, and for physiological activation of the transfected cells by T cell receptor ligation. Using these protocols to screen candidate constructs, we generated a panel of efficient retroviral expression vectors for antigen-regulated gene expression in T cells. These vectors are now being used to express IL-2 in murine tumor-specific CTL, and the transduced CTL are being evaluated for enhanced engraftment, persistence, and therapeutic efficacy *in vivo*. As an initial approach toward the identification and isolation of human tumor-specific CTL, we are investigating T cell responses directed against fusion proteins generated by chromosomal translocation. There are several human leukemias in which chromosomal translocation results in a chimeric gene expressing a novel fusion protein that might elicit a CTL response, including CML (the BCR-ABL gene), AML M2 (the AML1-ETO gene), and pre-B ALL (the E2A-PBX1 gene). The ability to isolate and expand leukemia-specific CTL directed against these fusion proteins, coupled with the ability to augment CTL efficacy by retrovirus-mediated gene transfer, might lead to the development of adoptive immunotherapy protocols for the effective treatment of these diseases.

R 508 RECOMBINANT P53 ADENOVIRUS (rAd/p53) EFFECTS ON PROLIFERATION OF HUMAN TUMOR CELL LINES

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Mutations in the p53 gene are the most frequent genetic alteration in human cancer, and loss of p53 function can lead to the uncontrolled growth observed in tumors. Replacement of wild-type p53 function has been shown to suppress the tumorigenic phenotype of malignant cells, suggesting a role for p53 gene therapy in the treatment of cancer. Consequently, a series of replication defective recombinant p53 adenovirus (rAd/p53) constructs has been developed to deliver p53. To test the in vitro effects of rAd/p53, a series of 28 human tumor cell lines were evaluated for endogenous p53 expression, adenovirus infection with rAd/βgal, and cellular proliferation after infection with rAd/p53. Results with rAd/βgal indicate differences in βgal expression among human colon, lung, liver, breast, and ovarian tumor cell lines. Data from ^{3}H-thymidine incorporation studies with rAd/p53 and rAd/βgal demonstrate p53-specific dose-dependent effects in several p53^{null} and p53^{mut} cell lines. No specific effects of rAd/p53 were observed on cell lines with wild-type p53. These initial data provide a basis for ongoing tumorigenicity studies with rAd/p53 and support a role for p53 adenovirus gene therapy in the treatment of tumors deficient for p53.

R 509 TOXIN GENE-LuIII PARVOVIRUS VECTOR FOR POTENTIAL CANCER THERAPY, Ian H. Maxwell and Françoise Maxwell, University of Colorado Cancer Center, Health Sciences Center, Denver, CO 80262

We have previously demonstrated ablation of specific cell types by regulated expression of the diphtheria toxin A-chain (DT-A) gene (I.H. Maxwell, L.M. Glode, F. Maxwell, Cancer Res. 51: 4299 (1991)). We have also constructed recombinant derivatives of the autonomous parvovirus, LuIII, capable of transient transduction of reporter genes (I.H. Maxwell et al., Human Gene Therapy 4: 441 (1993)). We plan to use a LuIII vector for toxin gene transduction into tumor cells, as a therapeutic approach. In initial experiments we constructed recombinant LuIII plasmids with the DT-A gene substituted for either the entire viral coding sequences or for just the capsid sequences. DT-A was thus placed under transcriptional control of the viral P4 or P38 promoter, respectively. Co-transfection of either plasmid into 324K or COS cells, together with the pSVLu helper plasmid, resulted in abundant production of virions containing the Lu-DT-A recombinant genome (detected by PCR with DT-A primers after extensive DNase treatment). However, neither virus preparation caused significant killing of fresh recipient cells even though infection with the P4 driven DT-A virus was demonstrated by an infected cell hybridization assay. Experiments are underway to try to explain the rapid accumulation and packaging of apparently inactive Lu-DT-A genomes in the transfected producer cells and to devise conditions for maintaining toxic activity.

R 510 RIBOZYMES SELECTIVELY CLEAVE MUTANT H-*RAS* ONCOGENE RNA OVER WILD-TYPE WITH >1000-FOLD DISCRIMINATION

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Ribozymes (Rbzs) hybridize to and enzymatically cleave complementary sequences of cellular RNA. Ribozyme therapeutic approaches offer one of the only means to selectively inhibit dominant mutant oncogenes without affecting the normal cellular counterparts. We have confirmed a report in which phosphorothioate antisense oligomers showed a five-fold discrimination between a point mutated 12th codon of H-*ras* and wild-type-ras target sequence(1). The discrimination of antisense oligomers was limited by the difference in thermodynamic stability between the hybrids formed between mutant and WT sequences (1). This same 12th codon mutation in H-*ras* creates a consensus GUC hammerhead (HH) Rbz target site, while the WT GGC sequences is not predicted to be a hammerhead site(2). Due to the sequence requirement for Rbz cleavage, a Rbz targeted to this site is predicted to have greater discrimination between mutated and WT RNAs than antisense molecules that rely on differences in thermodynamic stability. We have synthesized a series of Rbzs with nuclease resistant 2'-O-methyl arms targeted against this 12th codon mutant *ras* gene. Cleavage reactions in buffer systems with short synthetic target RNA show that these Rbzs cleave the mutant target at least 1000-fold faster than the wild-type target. Verification of this specificity in tissue culture cells is in progress.

1. Monia, B. P.,*etal* (1992). Selective Inhibition of Mutant Ha-*ras* messenger RNA Expression by Antisense Oligonucleotides. Journal of Biological Chemistry, 267(28), 19954-19962.
2. Kashani-Sabet, M.etal. (1992). Reversal of the Malignant Phenotype by an Anti-*ras* Ribozyme. Antisense Res. and Dev., 2, 3-15.

R 511 BIORESONANCE-INDUCED TUNNELING IN SERUM ALBUMIN IN CANCER TREATMENT, O.V. Zhalko-Titarenko, V.V. Liventsov, Institute of Experimental Pathology, Oncology and Radiobiology of the Ukrainian AS, Kiev, Ukraine, and G. Lednyiczky, Applied Logic Laboratory, Budapest, Hungary.

Owing to substantially cooperative character of the flexibility of protein/water system, the possibility of tunneling of atom groups of a protein between the local minima of its conformational potential can exceed activated transfer even at ambient temperature. The secondary structure of human serum albumin (HSA) is readily modified with its complex formation with low molecular weight proteins (LMWP), which are synthesized in patients with cancer. The population of various conformational domains in HSA in healthy people and patients with cancer is measured by IR spectroscopy and the shape of HSA conformational potential in physiological and pathological cases is calculated. The influence of ultra-low energy electromagnetic field on the possibility of atom tunneling across conformational potential of HSA is estimated by using a modified polaron model. HSA is considered as a system of coupled oscillators with strong fade. Various anharmonic effects are accounted for the complex formation of HSA with LMWP. Bioresonance interaction is shown to bring local minima of the HSA conformational potential closer and thus increase the possibility of atom tunneling. The conditions under which these interactions can restore the physiological conformation of patients with cancer are estimated.

Late Abstract

BCR-ABL-INDUCED ONCOGENESIS IS MEDIATED BY DIRECT INTERACTION WITH THE SH2 DOMAIN OF THE GRB-2 ADAPTOR PROTEIN. Mikhail L. Gishizky, Lawrence A. Quilliam, Larry D. Cripe, Craig H. Bassing, Zonghan Dai, Nanxin Li, Andreas Batzer, Kelly M. Rabun, Channing J. Der, Joseph Schlessinger and Ann Marie Pendergast,. SUGEN, Inc. 515 Galveston Dr., Redwood City, CA 94063, Department of Pharmacology, New York University Medical Center, New York, New York 10016, Department of Pharmacology, Duke University Medical Center, Durham, North Carolina 27710.

BCR-ABL is a chimeric oncoprotein that exhibits deregulated tyrosine kinase activity and is implicated in the pathogenesis of Philadelphia chromosome (Ph1)-positive human leukemias. Sequences within the first exon of BCR are required to activate the transforming potential of BCR-ABL. The SH2/SH3 domain-containing GRB-2 protein links tyrosine kinases to Ras signaling. We demonstrate that BCR-ABL exists in a complex with GRB-2 in vivo. Binding of GRB-2 to BCR-ABL is mediated by the direct interaction of the GRB-2 SH2 domain with a phosphorylated tyrosine, Y177, within the BCR first exon. The BCR-ABL-GRB-2 interaction is required for activation of the Ras signaling pathway. Mutation of Y177 to phenylalanine (Y177F) abolishes GRB-2 binding and abrogates BCR-ABL-induced Ras activation. The BCR-ABL (Y177F) mutant is unable to transform primary bone marrow cultures and is impaired in its ability to transform Rat1 fibroblasts. These demonstrate that GRB-2 not only functions in normal development and mitogenesis but also plays a role in oncogenesis. Thus disruption of this GRB2 interaction may have clinical utility in oncology.