TUMOR PROGRESSION AND METASTASIS

Isaiah J. Fidler and Garth L. Nicolson, Organizers

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Plenary Sessions Page

Poster Sessions

Tumor Progression and Diversification: Genetic Aspects

Q 001 MOLECULAR GENETICS OF HUMAN B AND T CELL NEOPLASIA. Carlo M. Croce, The Wistar Institute, 3601 Spruce Street, Philadelphia, PA. 19104

Sequence analysis of the breakpoints involved in the translocations of non-Burkitt B-cell tumors has also provided evidence that in most cases the chromosome translocations occur at the pre-B-cell stage of differentiation during the process of VDJ joining and that the VDJ recombinase is responsible for the translocation by catalyzing the joining of the involved chromosomes. Three observations indicate that this is the case: (1) in the great majority of non-Burkitt lymphomas, the translocation breakpoints involve the 5' region of a J segment; (2) extra nucleotides (N regions) are detected at joining sites in both the t(11;14) and the t(14;18) chromosome translocations; and (3) heptamer and nonamer signal sequences, separated by a spacer of 12 nucleotides, that closely resemble those involved in physiologic VDJ joining, occur on chromosomes 11 and 18 near breakpoints. Thus one can speculate that in a rare B-cell, the recombinase mistakenly joins a heavy chain J segment to a cellular prot-oncogene insted of the proper immunoglobulin gene segment, leading to oncogene deregulation.

Q 002 REGULATION of METASTATIC CELLULAR SUBPOPULATIONS in MALIGNANT TUMORS, George Poste, Smith Kline & French Laboratories, Phildelphia, PA 19101

The concept that tumors contain multiple subpopulations of cells that differ in a wide variety of phenotypic characteristics is well established. Variation in the invasive and metastatic properties of different cellular subpopulations and in their responses to various therapeutic modalities are now recognized as major obstacles to the effective therapy of neoplastic disease. Virtually nothing is known about the mechanisms by which cellular diversity is generated within malignant tumors or the kinetics of phenotypic diversification in tumors arising in different cell lineages. This in part reflects the substantial technical and logistical problems associated with the isolation, characterization and comparison of multiple clonal subpopulations involved in the analysis of these phenomena. Also, the high level of phenotypic instability displayed by many tumor cells imposes additional complexity on experimental effort to correlate specific phenotypic traits analyzed in short-term in vitro assays with behavioral traits such as metastasis that must of necessity be assayed in longer-term assays in vivo.

Data will be presented on the use of clonal analysis to monitor the sequential emergence of tumor cell clones with different metastatic properties during progressive growth of transplantable and autochthonous animal tumors. These experiments indicate that tumor progression is accompanied by the emergence of tumor cell clones that generate progeny with variant phenotypes at higher frequencies than clones isolated during the early stages of tumor evolution.

The high frequencies with which phenotypic variants are generated in many tumor cell populations in vitro and in vivo suggests that epigenetic mechanisms are involved in the genesis of cellular diversity. Resolution of the respective contributions of genetic and epigenetic mechanisms is hindered, however, by the lack of information about the specific genomic loci involved in regulating clinically important tumor cell traits such as antigen expression, drug resistance and complex multifactorial traits such as metastasis.

Q 003 CONTROL OF TUMORIGENIC EXPRESSION IN HUMAN CANCER CELLS BY SINGLE CHROMOSOME TRANSFER. Eric J. Stanbridge, Department of Microbiology and Molecular Genetics, University of California, Irvine, CA 92717.

Somatic cell hybridization experiments have clearly demostrated the control of tumorigenic expression by genetic regulatory elements derived from normal cells. A combination of cytogenetic and molecular studies (restriction fragment length polymorphism analyses) have implicated specific chromosomes whose presence in the hybrid cell is required for control of tumorigenic expression.

We have now developed techniques for the transfer and selective retention of single human chromosomes from normal cells into cancer cells via microcell transfer. We have further shown that the tumor-forming properties of both HeLa and Wilms' tumor cells are completely suppressed by the introduction of a single copy of chromosome 11 derived from human diploid fibroblasts.

Strategies for cloning and characterizing putative tumor-suppressor genes on chromosome 11 and other chromosomes will be discussed.

Q 004 CYTOGENETIC DIVERSITY IN PRIMARY HUMAN TUMORS Sandra R. Wolman, Patricia M. Camuto, Mary Ann Perle, Department of Pathology, New York University School of Medicine, New York, NY 10016

Direct cytogenetic analysis of fresh human tumor tissue demonstrates a wide variety of karyotypic alterations and often includes normal diploid cells. Short-term cultures, in contrast, display diversity over a narrower range apparently dependent on adaptability of different cells to growth in culture. DNA content studies by flow cytometry (FCM) provide a more comprehensive view of the entire tumor cell population but are less specific than karyotypic analyses. Examples based on studies of breast cancer, renal carcinoma and tumors of the central nervous system (CNS) illustrate several major problems. 1) Results vary with the tissue of origin: primary cultures from breast are almost uniformly diploid, while renal tumors are near-diploid, mosaic, and show clonal aberrations, and CNS tumors are heterogeneous; some diploid, some near-diploid and some highly aneuploid. 2) Results after short-term culture are selective, representing subpopulations from the heterogeneous cells that are detected on direct analysis of fresh tumors by cytogenetics or FCM. 3) Evidence from all three tumor types supports the interpretation that cytogenetically normal diploid cells constitute part of some tumor populations, and may be better adapted to routine growth in culture than aneuploid subpopulations from the same primary tumors.

Regional differences within tumors can be explored and genetic heterogeneity demonstrated by molecular probes and by multiple tumor sampling for FCM. Correlation of histologic grading with genetic analyses and clinical events is necessary for prognostication. It is not yet clear whether prognosis depends on the dominant population of the primary tumor or alternatively should be influenced by detection of small aneuploid subpopulations.

Tumor Progression and Diversification: Host Aspects

Q 005 THE ROLE OF INFLAMMATORY CELLS IN TUMOR PROGRESSION AND DIVERSIFICATION: HOST ASPECTS, Gloria H. Heppner, Wei-Zen Wei, Michigan Cancer Foundation, 110 E. Warren Ave., Detroit, MI 48201.
Tumor progression is the result of multiple cellular interactions among heterogeneous tumor populations and between tumor and host cells. Our laboratory is investigating the role of one class of host component in this process, namely the inflammatory cell infiltrate. Data will be presented from two different mammary tumor systems that illustrate the involvement of macrophages and of NK cells, respectively. The first system is a series of tumor subpopulation lines derived from a single mouse mammary tumor and which differ in ability to metastasize. Although we have not found evidence of enhanced inherent instability in metastatic vs. nonmetastatic subpopulations, we have found that metastatic cells are more sensitive to an exogenous mutagen. We have identified a possible source of exogenous mutagen in the tumor environment, namely tumor infiltrating macrophages. Activated macrophages (both peritoneal and tumor associated) are capable of in vitro induction of thioguanine-resistant variants in mammary tumor line 66. The mutation is due to alteration at the HGPRT locus. The stability of the macrophage-induced mutation is comparable to that induced by classic mutagens. Mutagenicity is blocked by inhibitors of active oxygen metabolites. Our results suggest that tumor heterogeneity, and consequently progression, can be fueled by macrophage factors. The second system is the hyperplastic aveolar nodule (HAN) line, C4, and C4 tumors that develop spontaneously within HAN implants. We have found that active NK cells accumulate in the preneoplastic HAN tissues and that their relative number and activity wanes and NK suppressor activity rises as tumors develop. Since NK cells also produce hydroxyl radicals, it is possible that tissue infiltrating NK cells are directly involved in the generation of variant, tumorigenic populations from preneoplastic cells. Alternatively, NK cells secrete a number of cytokines including interferon, interleukin-1 and colony stimulating factor which may activate infiltrating macrophages and in that way trigger the machinery of tumor progression.
Supported by NIH grant CA 27437 and by a grant from Concern Foundation.

Q 006 DNA METHYLATION PATTERNS AND TUMOR HETEROGENEITY, Peter A. Jones, Lois A. Chandler, Hamid Ghazi, Thomas Ahlering, Louis Dubeau and Tim Kautiainen, Urological Research Laboratory, USC Cancer Center, Los Angeles, CA 90033.
One part of the multilevel mechanism controlling gene expression in eukaryotic cells appears to be the methylation of specific cytosine residues in DNA. DNA methylation patterns are tissue-specific and are copied with a high degree of fidelity in normal cells during development. Aberrations within this information controlling system might be important during the process of tumor progression and metastasis and we have therefore investigated aspects of methylation control and methylation patterns in tumorigenic cells. The levels of maintenance DNA methyltransferase enzyme in tumorigenic cells were found to be much elevated compared to the levels found in non-tumorigenic counterparts, suggesting that alterations within the copying mechanism might exist within tumor cells. We examined a series of human cells with known tumorigenic potentials and found that the methylation patterns of normal cellular genes and cellular proto-oncogenes including the c-Ha-ras gene were remarkably heterogeneous within these cells. A clonal analysis of several human cell types including the EJ bladder carcinoma cell line showed that this heterogeneity extended even to individual alleles of the c-Ha-ras genes within the cell. Thus, individual copies of the c-Ha-ras gene which could be identified by the presence of restriction fragment length polymorphisms were differentially methylated implying a far greater degree of heterogeneity in methylation patterns than previously thought. A nude mouse model for metastasis of the EJ bladder carcinoma cell line was developed by the direct inoculation of EJ cells into the mouse urinary bladder. The primary tumors and lung metastases arising from these inoculations were grown in culture and the methylation levels and patterns of the ras gene probed. These experiments showed that the ras gene in the primary and secondary tumors was further demethylated. The possibility that altered methylation patterns contribute to tumor cell heterogeneity will be discussed.

Q 007 CANCER METASTASIS, T CELL IMMUNITY AND IMMUNOTHERAPY IN A MODEL SYSTEM, Volker Schirrmacher, Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, D-6900 Heidelberg, FRG.
We will review our experience with the highly metastatic mouse tumor ESb and focus on the aspects of T cell recognition and immunotherapy. In spite of the fact that the tumor ist one of the most malignant animal tumors, inoculation into the pinna enables a strong T cell mediated host immunity which is capable of rejecting up to 5 x 10^4 life ESb tumor cells. Upon metastases of this tumor from a s.c. inoculation site at the back, host T cell responses are induced which are incomplete and do not lead to rejection of the tumor. Instead the tumor is able to develop selective immune escape variants. Upon modification of the tumor cell with Newcastle Disease Virus the incomplete anti tumor cytotoxic T cell response can be activated. Such virus modified tumor cells could be shown to be an effective vaccine to be used for active specific postoperative immunotherapy of micrometastases.

Q 008 **UTILIZATION OF GENE TRANSFECTION TO STUDY ASPECTS OF THE BIOLOGY OF TUMOR PROGRESSION AND METASTASIS.** **Carol Waghorne[1], Martin L. Breitman[1], Shan Man[1] Bruce E. Elliott[2] and Robert S. Kerbel[1]. Mt. Sinai Hospital Research Institute[1], Toronto Ontario, Canada and The Dept. of Pathology, Queen s University, Botterell Hall, Kingston, Ontario, Canada[2].**
Gene transfer techniques have created new and unique opportunities to study a variety of problems related to tumor progression and metastasis We will review our latest findings and present results which highlight the potential of this approach as well as some possible pitfalls. We have been studying the effects of transfected oncogenes on the metastatic potential of a spontaneous mouse mammary adenocarcinoma SP1 which is normally non-metastatic but highly tumorigenic when injected subcutaneously into syngeneic CBA/J mice. Virtually every (>90%) SP1 clone transfected with the activated human H ras oncogene gave rise to lung nodules in at least 1/5 animals. While metastases were never observed following subcutaneous inoculation of the parental SP1 cells or 40 independent SP1 clones, 20% of the control pSV_2neo transfected clones metastasized to the lungs of some animals. In addition, another 20% of the transfected SP1 clones actually exhibited a significant loss of tumorigenic ability in normal CBA/J mice, but not in athymic nude mice, indicating acquisition of significant immunologic changes. This was correlated with a high level of $H\text{-}2D^k$ antigens in these clones, unlike the SP1 parent. This suggested that the $CaPO_4$ mediated gene transfection procedure itself could affect high frequency changes in gene expression within the genome of the recipient cell. We have further evidence which suggests that $CaPO_4$ treatment alone can elicit at least some of these changes (ie 25% of $CaPO_4$ treated clones were metastatic). Because of the limited number of copies inserted and the random nature of the insertions, we found that Southern analysis of tumor cells recovered in vivo could be exploited as a novel genetic tool to study selection and clonality of tumor growth. In conclusion, although $CaPO_4$ mediated gene transfection is a powerful tool to help analyze many aspects of the nature of malignancy in vivo, the procedure itself may cause high frequency changes in gene expression, and this may complicate interpretation of tumorigenicity and/or metastasis results that are subsequently obtained.

Regulation of the Metastatic Phenotype

Q 009 GENETIC MECHANISMS AFFECTING THE METASTATIC PHENOTYPE, Rene Bernards and Robert A. Weinberg. Whitehead Institute, Cambridge, MA 02142. Considerable evidence exists to suggest that activated cellular oncogenes play a crucial part in the initial transformation of normal cells into tumor cells. The role of activated oncogenes in the later stages of tumor development, however, is less well understood. The most compelling evidence that suggests that oncogenes are indeed involved in the progression of certain tumors to a more malignant state comes from clinical studies of neuroblastoma. In these studies it was shown that advanced stages of disease (i.e. progressive degrees of metastatic spread) correlates with increased amplification of the N-myc oncogene. We have concentrated on this neuroblastoma model in an attempt to understand the changes that take place in neuroblastoma cells when the N-myc gene becomes over-expressed. To do this, we used a rat neuroblastoma cell line into which we introduced the N-myc gene in these cells was found to dramatically increase their metastatic ability, to cause down-modulation of class I histocompatibility antigen expression, and to greatly increase the in vivo growth rate. Evidence will be presented to show that some of the effects of the N-myc oncogene on tumor cells are cell-type specific.

Q 010 MHC GENES, ONCOGENES AND ANTIGENS CONTROLLING THE METASTATIC PHENOTYPE, Michael Feldman and Lea Eisenbach, Department of Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel

The recognition by CTL of cell surface epitopes is restricted by products of genes coding for class 1 antigens of the MHC. If a tumor-associated epitope elicited CTL restricted by one but not by another class 1 antigen on disseminating cells of a metastatic tumor, then this would determine the probability of metastasis formation as a function of their escape from the host's T cell immune elimination. Indeed, we found that metastatic clones of the 3LL tumor expressed the H-2D^b antigens, but hardly the H-2K^b, whereas the nonmetastatic clones expressed both H-2K^b and H-2D^b. In fact, the metastatic phenotype was inversely correlated with the H-2K^b/H-2D^b ratio. This was demonstrated to be causally related to the metastatic phenotype, since expression of H-2K^b following H-2K^b gene transfection resulted in abolishment of reduction of the metastatic competence. Essentially similar results (abolishment of metastatic phenotype following H-2K transfection) were obtained with the T10 sarcoma. The induced expression of H-2K genes was associated with the acquisition of H-2K restricted immunogenicity. The normal expression of MHC genes by nonmetastatic cells was correlated with the expression of the c-fos proto-oncogene. Since the treatment of H-2K$^-$, H-2D$^+$ metastatic cells with gamma interferon, resulting in the induced expression of H-2K^b, was preceded by a transient induction of c-fos transcripts, it appeared that the latter might be causally related to the control of MHC expression. This causal relationship was experimentally demonstrated in studies in which transfection of H-2K$^-$ metastatic cells with v-fos was followed by expression of H-2K^b genes. Thus, the c-fos proto-oncogene, controlling the expression of class 1 antigens, controlled their metastatic phenotype.

The H-2K determined immunogenic capacity, decreasing the probability of metastasis formation, reflected only part of the properties differentiating metastatic from nonmetastatic phenotypes. In addition, we found that the metastatic variants manifested a plasma membrane-bound protein tyrosine kinase. Its properties were similar to kinases which characterize receptors for growth factors or oncogene products. In fact, such metastatic cells, but not nonmetastatic variants, expressed a fms-related oncogene which could code for the tyrosine kinase. Whether we face a proto-oncogene coding for a receptor to a factor which directs growth of the neoplastic cells at the metastatic target organ is an inviting possibility.

Q 011 RAS^H ONCOGENES AND METASTASIS, Ruth J. Muschel, Laboratory of Pathology, NCI, Bethesda, MD.

This work has focused upon the effect of ras^H oncogene transformation on metastasis. We have found that NIH 3T3 cells transformed by the ras^H oncogene are metastatic in nude mice. In contrast, NIH 3T3 transformed by mos or sarc are not metastatic under the same conditions. We tested the generality of this observation by introducing ras^H into rat embryo fibroblasts (REF). REF transformed by ras^H and myc, or by ras^H alone (ras cotransfected with pRSVneo or ras^H linked to an enhancer) were also highly metastatic in nude mice. Thus, the ras^H oncogene induces metastasis as well as tumorigenicity, However, REF transformed by ras^H plus AdE1a are not metastatic in nude mice. These cells were tested for type IV collagenase secretion. The transformed REF which were metastatic secreted high levels of the enzyme but REF transformed by ras^H plus AdE1a had only baseline levels. AdE1a may suppres metastasis by reducing the intrinsic aggressiveness of the cells. Becuase the REF are initially diploid, we wished to investigate their karyotyes after transformation, tumor formation and metastasis. Surprisingly, the cells transformed by ras^H alone remained either diploid or near diploid after transformation and after metastasis. Many but not all of the REF transformed by ras^H plus myc were also diploid and were unaltered in metastasis. Thus, chromosomal rearrangements or genomic instability seem an unlikely explanation for the generation of metastasis in these cells.

Implantation and Growth

Q 012 INVASION AND METASTASIS OF FR3T3 CELLS TRANSFECTED WITH BPV-1 DNA, Marc Mareel, Frans Van Roy, Peter Coopman, Jin Gao, Chris Dragonetti, Ludwine Messiaen and Walter Fiers, Lab for Experimental Cancerology and Lab for Molecular Biology, State University of Ghent, B-9000 Ghent, Belgium; Chinese Academy of Basic Medical Sciences, Beijing, China.

FR3T3 cells transfected with bovine papilloma virus type-1 (BPV-1) DNA were reported to combine a normal morphotype in culture with a high metastatic capability after injection into syngeneic Fischer rats[1]. This provided us with the opportunity to study acquisition of invasion and of metastatic capability in absence of the loss of growth control that occurred after transfection with transforming genes[2]. Low passage (8 to 15) FR3T3 cells before and after transfection with the complete BPV-1 genome or with the BPV-1 transforming region (pV69), high passage FR3T3 cells, and tumor-derivatives (TD) of low passage FR3T3 cells, of pV69-transfectants, and of BPV-1-transfectants were tested for invasiveness and metastatic capability. All cell types produced tumors after s.c. or i.p. injection of 5 x 10^6 cells. Ranking of latency periods was : TD-lines < high passage FR3T3 cells < BPV-1-transfectants < pV69-transfectants < low passage FR3T3 cells. All tumors were invasive, in agreement with the results obtained by confrontation of cells with embryonic chick heart in organ culture. Cells from primary REF cultures from which FR3T3 cell lines were established were non-invasive in vitro[2], but neither in vitro nor in vivo FR3T3 cells could be scored non-invasive. We, therefore, concluded that the invasive phenotype was acquired either together with or before immortalization of REF cells, in agreement with previous observations[2]. With C3H mouse lens epithelial cell lines we were able to mark the transition between invasiveness and non-invasiveness in the assay in vitro, but tumors in syngeneic mice were unvariably invasive. Variations in metastatic capability were observed after s.c. or i.p. injection of transfectants or of their TD-lines. The frequency of metastasis was 7/20 for pV69-transfectants, 3/4 for BPV-1-transfectants, and 8/8 for BPV-1-TD cells. After s.c. implantation of a cellular aggregate in the tail all tumors produced by transfectants as well as by untransfected low passage FR3T3-TD cells were metastatic. This confirms the conclusion from experiments with KiMSV-transformed C3H mouse cells that growth in the tail enhances the metastatic capability of a cell population[3]. From the present experiments with BPV-1 DNA and from previous work with immortalizing and transforming genes[2] the following picture emerged : FR3T3 cells in culture rapidly acquire invasiveness. Metastatic capability is acquired at a slower rate under the influence of, as yet, unknown factors. Transfection with a number of various and apparently unrelated gene sequences as well as passage in vivo speeds up the appearance of the metastatic phenotype.

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Q 013 USE OF A RECONSTITUTED BASEMENT MEMBRANE TO STUDY THE INVASIVENESS OF TUMOR CELLS, Y. Iwamoto, A. Albini, R. Reich, K. Romisch, E. Thompson, J. Graf, Y. Yamada, R.N. McEwan and G.R. Martin, LDBA, NIDR, NIH, Bethesda, MD, 20892, The Upjohn Company, Molecular Biology Research, Kalamazoo, MI 49001.

We have used an extract of basement membranes which can be reconstituted into a biologically active gel matrix composed predominantly of collagen IV, laminin, nidogen, and heparan sulfate proteoglycan, to study mechanisms involved in tumor cell invasion. When layered onto a porous filter in a Boyden chamber, the gel forms a barrier to the passage of normal cells. Malignant cells rapidly cross this layer when laminin or another chemoattractant is used to stimulate cell migration. The cells which cross the matrix barrier can be quantitatively recovered for further study. A variety of human and animal tumor cells have been studied in this system and we find a high correlation between their invasiveness in vitro and the ability to form metastasis in vivo. Further, the cells penetrating through the barrier are more invasive and metastatic than the original cell line. We have used this in vitro invasion assay to test for factors which might inhibit tumor cell invasion. Collagenase IV is produced by malignant cells and is thought to be required for invasion. Indeed, we find that inhibitors of this enzyme reduce tumor cell invasiveness. Because laminin is a chemoattractant for metastatic cells, synthetic peptides corresponding to sequences from the B1 chain of laminin were tested for their ability to compete with laminin and block cell migration. A chemotactic peptide (CDPGYIGSR) which corresponds to residues 925 to 933 in the B1 chain of laminin, blocks the invasiveness of malignant tumor cells. These data suggest that this assay can be utilized to study the invasive activity of tumor cells and those factors that may inhibit the spread of malignant cells.

Q 014 **HEPARANASES AND TUMOR METASTASIS**, Motowo Nakajima, Tatsuro Irimura and Garth L. Nicolson, Department of Tumor Biology, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston TX 77030.

The successful penetration of endothelial basement membrane is an importance process in hematogenous tumor metastasis. Heparan sulfate (HS) proteoglycan is a major constituent of endothelial basement membrane, and we have found that HS degradative activities of B16 melanoma sublines correlated with their lung-colonizing potentials (1). Melanoma HS-degrading endoglycosidase is a unique endo-β-glucuronidase (heparanase) capable of specifically cleaving HS (2). Heparanase activity was detected in all cultured human malignant melanoma cells tested using a solid-phase substrate of partially N-desulfated N-[^{14}C]acetylated HS crosslinked to agarose gel beads via one covalent linkage (3,4). The treatment of B16-BL6 melanoma cells with heparanase inhibitors that have minimal other biological activities, such as N-acetylated N-desulfated heparin and N-resulfated N- and O-desulfated heparin, resulted in significant reductions in the numbers of experimental melanoma lung metastases in C57BL/6 mice (5). These results indicate that heparanase plays an important role in melanoma metastasis. Heparanases were purified from mouse B16-BL6 and human A375 melanoma cells by use of heparin-Sepharose, concanavalin A-Sepharose, and N-acetylated N-desulfated heparin-Sepharose affinity column chromatography. The human and mouse melanoma heparanases are of M_r ~96,000, as determined by SDS-polyacrylamide gel electrophoresis. We further studied the heparanase substrate specificity using HS proteoglycan purified from EHS tumor and found that the melanoma heparanase cleaves β-D-glucuronosyl-N-acetylglucosaminyl linkages. Since human malignant melanoma tissues have high heparanase activities and the heparanase is released from melanoma cells, we tested sera from malignant melanoma patients and normal adults for heparanase. The average serum heparanase activities of melanoma patients ($n = 35$) and normal adults ($n = 15$) were 3.72 and 1.98 U/ml serum, respectively ($P<0.05$). The source of heparanase activity in the normal adult serum is possibly platelets and other blood cells because heparin-degrading activity was also detected. The sera from the patients having metastases in lymph nodes, liver, and lung showed more than 4 fold higher heparanase activities than normal controls ($P<0.001$). Supported by NCI grants RO1-CA41524 to M.N. and RO1-CA42346 to G.L.N. and a grant from the National Foundation for Cancer Research to G.L.N..

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Invasion and Dissemination

Q 015 GROWTH REGULATION IN COLON CARCINOMA, Russell G. Greig, Mario Anzano, Deborah Trainer and George Poste, Department of Cell Biology, Smith Kline & French Laboratories, Philadelphia, PA 19101.
The goal of our studies is to examine the molecular events underlying the pathogenesis of colorectal carcinoma and to identify new pharmacological targets for the treatment of this neoplasm. In a set of initial investigations we have characterized the biological behavior of 20 human colon carcinoma cell lines and correlated these properties with expression of specific gene products, including (proto)oncogenes, growth factors and growth factor receptors. Tumorigenicity, metastatic capacity and histological grading were determined by inoculating colon carcinoma cell lines via several routes (iv, sc, ifp and is) and at multiple doses into nude mice. Expression of the colon carcinoma-associated markers CEA and CA 19.9 was determined in culture supernatants. Growth factor production (TGF-α, TGF-ß and EGF) was monitored using conventional assays and the ability of each cell line to proliferate in soft agar was also examined. Oncogene expression was investigated by extracting mRNA from both monolayer cultures and tumor xenografts followed by Northern blot analyses. Each colon cell line could be classified into one of three categories depending upon its tumorigenicity in nude mice. Class I lines were highly tumorigenic whereas Class II populations formed slower growing tumors. Class III lines were non-tumorigenic. No cell line was consistently metastatic. The oncogenes c-myc, H-ras, K-ras, N-ras, myb and p53 were expressed in nearly all cell lines. In contrast, abl, src and ros were not detectable. Where determined, the levels of oncogene mRNA expression varied from 100-1000 copies per cell (c-myc) to 10-100 copies (H-ras) and < 10 copies (fos and myb). Southern analysis failed to detect gross rearrangements, deletions or amplifications of the oncogenes examined and the pattern of oncogene expression was essentially unaltered when cell lines were propagated as xenografts in nude mice. Several colon cell lines (5/11) secreted TGF-α/EGF activity and 6/11 produced TGF-ß. Nearly all colon cell lines examined expressed receptors for TGF-α/EGF. The best in vitro predictor of tumorigenicity was colony formation in soft agar. There was no detectable correlation between malignant properties in nude mice and oncogene expression, production of colon-associated antigens, morphology, doubling time in vitro or growth factor production. The availability of this well characterized panel of colon cell lines will be extremely useful in examining mechanisms of growth regulation in normal and neoplastic colon.

Q 016 ON THE ROLE OF TUMOR CELL LECTINS IN METASTASIS, Avraham Raz and Reuben Lotan, Department of Cell Biology, The Weizmann Institute of Science, Rehovot, 76100, Israel and Department of Tumor Biology, The University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030, USA.
The propensity of tumor cells to spread in the body and produce metastases is the major cause of death from cancer. Recognition and adhesion among the circulating tumor cells and between tumor and normal host cells lead to the formation of multicell emboli, a process causally related to metastasis. The molecular nature of the cell surface proteins mediating such processes has only recently been characterized. Among these is a group of molecules capable of binding with carbohydrate residues on adjacent cells, collectively known as endogenous lectins. Previously, we demonstrated that galactoside-specific lectins are present in a wide variety of human and murine tumor cells. The generation of mono- and polyclonal antibodies enalbed us to establish that the lectins are simultaneously exposed on the cell surface and localized within the cell's cytoplasm. The accumulated results prompted the notion that the cell surface expression of tumor cell lectins is related to neoplastic transformation and tumor progression. In order to establish the role of the cell surface lectins in metastasis, metastasizing B16 melanoma and UV2237 fibrosarcoma cells were incubated in vitro with the 5D7 anti-lectin monoclonal antibody. The treated cells were then injected into the tail vein of syngeneic mice and the development of metastases was examined thereafter. Such pretreatment resulted in a marked decrease (up to 90%) in the development of tumor colonies in the lung. Further studies have highlighted the role of the surface lectins in mediating cell-cell and cell-substrate interactions in vitro, pointing to the possible role of such sugar-binding proteins and their natural carbohydrate ligands in tumor cell dissemination.

Q 017 STUDIES ON THE GENETIC BASIS OF TUMOUR METASTASIS, David Tarin, Nuffield Department of Pathology, (University of Oxford), John Radcliffe Hospital, Headington, Oxford, OX3 9DU, England.
Techniques to examine the genetic disturbances involved in development of the metastatic phenotype in tumour cell populations are now becoming available. Approaches so far adopted in this laboratory include transfection of non-metastatic or weakly metastatic cells with cloned oncogenes or with total genomic DNA from highly metastatic cells as well as treatment of tumour cell populations with drugs known to modulate gene expression. In our studies transfection of non-metastatic tumour cells with the purified 6.6Kb activated ras oncogene did not confer metastatic capability on 3T3 fibrosarcoma cells, although it did enable them to colonise the lungs after intravenous inoculation. We therefore concluded that activation of this oncogene is not sufficient by itself to endow metastatic capability and that complementation by other groups of genes is almost certainly involved. Further transfection experiments using total genomic DNA from a highly metastatic human tumour cell line have resulted in marked augmentation of capability to colonise distant organs after intravenous inoculation mouse fibrosarcoma cells, together with development of capability to colonise many other organs distal to the lungs. Augmentation of spontaneous metastatic capability of mouse mammary carcinoma cells following transfection with DNA from highly metastatic mouse histiocytic sarcoma has also been observed in our experiments.

In other studies we have seen increase of spontaneous metastatic capability following treatment of tumour cells with 2'-deoxy 5-azacytidine and tetraphorbol acetate (TPA) which are agents known to affect tumour progression. Azacytidine is also known to alter gene expression and it is feasible that this is the mechanism of its action on some of the tumour cells that we have studied. These effects are heritable because they are seen several cell generations after inoculation of the treated tumour cells.

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Skin and Lymph Node Metastasis

Q 018 TUMOR PROGRESSION AND METASTASIS: CLINICAL IMPLICATIONS, Charles M. Balch, M.D. Anderson Hospital & Tumor Institute, Division of Surgery, 6723 Bertner Avenue, Houston, Texas 77030.
The role of regional lymph nodes in containing or delaying progression of metastases is as controversial as it is important. On the one hand, lymph nodes have been shown to serve as a temporary "filter" for metastatic tumors. Different tumor systems and especially different experimental conditions could indeed influence the relative biological behavior of metastatic tumor cells. On the one hand, both experimental and clinical evidence support a hypotheses that some metastases disseminate sequentially from regional to distant sites. On the other hand, there is evidence to the contrary that lymph nodes are merely indicators of disease, that growth in lymph nodes and distant sites are random events; as a consequence, surgical removal of these nodes will not increase the rate of cure. An alternative explanation is that the period of isolated lymph node metastases occurred prior to clinical detection of the primary tumor. The exact role of lymph nodes draining a primary tumor and the control of metastatic spread thus remains uncertain and several clinical trials are now being conducted to address this specific question. This includes a randomized clinical trial addressing the efficacy of elective (prophylactic) lymph node dissection in a targeted subgroup of melanoma patients (high risk for regional metastases, low risk for distant metastases). Determining this role is important, since the decision to surgically excise the regional lymph nodes must be based upon the estimated risk of lymph node metastases, and a biological proof that the process of metastases is sequential.

Q 019 LYMPHATIC METASTASIS, Ian Carr and Norman Pettigrew, University of Manitoba and St. Boniface General Hospital, Winnipeg, Manitoba, Canada R2H 2A6.

Lymphatic metastasis is an important mechanism in the spread of human cancer. Previous animal work has shown that cancer cells invade peripheral lymphatics by active movement of single cells, by movement of clusters of cells, and by induction of necrosis of the lymphatic endothelium. The cancer cells move singly or in clusters up the lymphatic trunk and settle in the subcapsular sinus. Once there they either migrate through the endothelium, or destroy it.

We have investigated the process of invasion, particularly invasion of lymphatic vessels in human colorectal cancer by EM, tissue culture, morphometry and flow cytometry. The invasion front is composed of poorly differentiated cells usually attached to the tips of neoplastic glands. The basal cytoplasm of these cells resembles the cytoplasmic processes seen in moving cells in vitro. The nuclear pattern at the invasive edge is morphometrically distinct, and is presently being examined for nuclear ploidy by flow cytometry. The invasive edge frequently shows extensive inflammatory change, and the lymphatic vessels are infiltrated by leucocytes. Some of the neoplastic cells move actively in tissue culture.

The findings suggest that invasion consists of invaginating movement of the tips of neoplastic glands, into tissue rendered abnormal by inflammatory changes. Such invaginating gland tips penetrate lymphatic vessels, and are carried off as clusters to the draining lymph node. This idea has some implications for any view of invasion and metastasis based on the behaviour of single cells. It is likely that future investigation will show significant variations between the invasive patterns of different types of human cancer.

Supported by the National Cancer Institute of Canada, and St. Boniface General Hospital Research Foundation.

Q 020 IN VIVO AND IN VITRO EFFECTS OF EPIDERMAL THYMOCYTE ACTIVATING FACTOR ON TUMOR GROWTH AND TUMOR CELL CHEMOTAXIS, D.N. Sauder, D. Guy, N. Damjii and F.W. Orr, Depts. of Medicine and Pathology, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5

Epidermal thymocyte activating factor (ETAF) has a multitude of immunoregulatory and pro-inflammatory properties. ETAF production can be altered by a variety of stimuli including ultraviolet radiation (UVR). UVR is also capable of inducing certain cutaneous tumors particularly fibrosarcomas in mice. This study was undertaken to evaluate the potential role of ETAF in neoplastic diseases. For this purpose, a UVR induced murine fibrosarcoma cell line was used. 5×10^6 tumor cells were injected subcutaneously (SC) into syngeneic mice followed by daily (SC) injections of ETAF or negative control. After 11 days, the animals were sacrificed and the tumors were dissected from the subcutaneous tissue and weighed. ETAF treated animals had a significant increase in tumor weight compared to control ($1.3 \pm .2$ vs $0.4 \pm .1$ $p < 0.01$). in vitro ETAF had no effect on tumor growth. Passive transfer experiments revealed that augmentation of tumor growth occurred when spleen cells from ETAF treated mice were transferred to naive recipients who were then challenged with tumor. Studies were also undertaken to determine the effect of ETAF on tumor cell chemotaxis. This revealed that ETAF was chemotactic for tumor cells (mean chemotactic activity 18 ± 2 vs $6 \pm 1 \pm 4.9$ for control). The results of this study indicate that ETAF can augment tumor growth in vivo and this is in part mediated by the generation of suppressor cells in ETAF treated mice and may also relate to the chemotactic effects of ETAF.

Urogenital Metastasis

Q 021 INTERACTIONS OF HUMAN CARCINOMA CELLS WITH EXTRACELLULAR MATRIX. R.J. Bernacki, K. Pavelic, M.A. Bulbulm Y.M. Rustum, M.J. Niedbala and K. Crickard*, Grace Cancer Drug Center, Roswell Park Memorial Institute, and Buffalo General Hospital*, Buffalo, New York 14263.
Our experience with culture dishes coated with extracellular matrix (ECM), produced by bovine corneal endothelial cells, has shown that ECM can serve as a biochemically complex, biologically relevant substrate, that supports primary epithelial (ovarian and urological) human tumor cell growth in vitro. Growth success for different ovarian and urological carcinomas (prostatic, bladder, kidney and testicular) following enzymatic digestion was compared after seeding fresh surgical explants onto ECM and plastic culture flasks. Tumor cells demonstrated rapid attachment to ECM with subsequent proliferation of tumor cell colonies. Approximately 80% of the surgical samples grew cell colonies on ECM. Ovarian, renal and prostatic had excellent success rates with bladder and testicular tumors being more difficult to culture. Poorly differentiated, metastatic renal, prostatic or ovarian carcinomas caused visual degradation of ECM. There appeared to be a correlation between the release of certain tumor hydrolases, such as β-N-acetylglucosaminidase, cellular degradation of ECM, and invasive potential. This model system is useful for establishing human tumor colonies in vitro and investigating their mechanisms of tissue invasion and drug sensitivity. (Supported by CA-13038 and CA-42898).

Q 022 SEX, STEROIDS AND CANCER, James H. Clark, Dept. Cell Biology, Baylor College of Medicine, Houston, Tx 77030
Most biologists would agree that the physiology of humans has not changed for the last 10,000 years. Therefore, we exist in a modern society with bodies that evolved and were adapted to cope with an environment of much earlier times. The purpose of this talk is to present current problems with sex hormones and cancer in the light of our evolutionary reproductive history. Topics to be covered include: 1) The loss of behavioral estrus during the reproductive cycle and its relationship to the development of reproductive tract and mammary cancer. 2) The role of the contraceptive pill in preventing cancer and other problems associated with inadequate reproductive cycles. 3) The protective effects of pregnancy and its relevance to our evolutionary past. 4) The role of progesterone in counteracting and controlling the actions of estrogen. 5) The menopause and its many problems as an artifact of civilization. 6) The causes of neoplasia during menopause. 7) The DES controversy. 8) The persistent estrus syndrome and its lack of relationship to human estrogen exposure.

The general conclusion will be that we have the knowledge and methods at hand to greatly modify and in many cases prevent deficiences and diseases that develop as a consequence of our inadequately adapted physiology. Whether this knowledge should be or can be used is a subject for debate.

Q 023 PROSTATE CANCER AND THE INVASIVE PHENOTYPE:NEW IN VIVO AND IN VITRO APPROACHES, James M. Kozlowski, Robert McEwan, Chung Lee, Harold Keer, John T. Grayhack and Adriana Albini, Department of Urology, Northwestern University Medical School, Chicago, Il. 60611 and *Laboratory of Developmental Biology and Anomalies, National Institute of Dental Research, Bethesda,MD 20892.

In terms of incidence, adenocarcinoma of the prostate is the second most common cancer among American males and approaches parity with colo-rectal cancer as the second most common cause of male cancer death. The majority of such patients present with locally invasive(Stage C) or metastatic(Stage D) disease at the time of diagnosis. Investigation of the invasive/metastatic phenotype as it applies to this neoplasm has been hindered by:(1)the scarcity of human prostate tumor systems amenable to in vitro analysis or in vivo propagation in nude mice and (2) the lack of a methodology capable of predicting the invasive potential of individual tumors. Our research efforts have focused on these perceived needs.

Previous studies have documented the utility of the intrasplenic(ISPL) injection assay with regard to assessing the invasive/metastatic potential of human tumor cell lines grown in athymic nude mice. Prostate cancer cell lines PC-3 and DU-145 were tested with this approach using 4-week old male Balb/c nude mice. Local tumors(spleen,body wall), invasive variants(diaphragm), and metastases(liver,lymph nodes,lung,ascites)were documented and established as sub-lines. The aggressive variants exhibited greater experimental(i.v.) metastasis than the parental tumor. With this approach, metastases were observed in 1/7 fresh prostate tumors.

Two in vitro techniques were used to expand the "library" of biologically diverse human prostate cancers. First, DU-145(low metastatic) was transfected with the EJ-ras oncogene using the plasmid vector pBR322 to produce 4 variants(DU-1, DU-1ASC,DU-EJ7,DU-EJ8)with high metastatic potential(ISPL injection assay). Control transfectants(neo, EC-ras) retained the indolent phenotype of DU-145. Second,epithelial/stromal fractions from benign(17) and malignant (7) fresh prostate specimens were separated(discontinuous Percoll gradient) and routinely established in finite(3-4 passages)tissue culture using serum-free media(WAJC404) plus essential growth factors,including bovine hypothalamus extract and cholera toxin.

Attachment to and degradation of the host basement membrane is an integral component of the metastatic cascade. Basement membrane biomatrix(MATRIGEL) derived from the EHS murine sarcoma was used to assess its impact on 1)the culture morphology of tumor cells and 2)their ability to penetrate a matrix-coated Nuclepore(12u) filter in a 5-hr chemoinvasion assay using a modified Boyden chamber. Invasive prostate tumors(established/fresh)demonstrated:1)prompt spreading with "filopodial" extensions;2)quantifiable penetration of the membrane barrier; and 3) a close correlation with in vivo metastatic capacity(ISPL injection assay).

Lung and Brain Metastasis

Q 024 AMF STIMULATED TUMOR CELL MOTILITY: USE OF DIFFERENT MATRIX RECEPTORS ON LAMININ VERSUS FIBRONECTIN, Lance Liotta, Giulia Taraboletti, Ulla Wewer, National Institutes of Health, National Cancer Institute, 9000 Rockville Pike, Bethesda, MD 20892

One of the least understood aspects of tumor invasion is tumor cell locomotion. Our recent identification of a tumor cell motility factor has opened a new approach to study the genetic control of tumor cell invasion. Human melanoma and breast carcinoma cells secrete a protein which binds to a cell surface receptor and markedly (up to 400 fold) stimulates locomotion by the producer cells. The factor is termed autocrine motility factor (AMF) and has been purified and partially characterized. One form is a 54 kDa protein which binds to a cell surface receptor ($Kd=5\times10^{-10}$ M) inducing alterations in membrane phospholipids and G proteins. The sequence of the first 19 NH_2 terminal amino acid residues of AMF has been determined. By comparison to protein data bases, this sequence is unique. Motility induction by AMF is not blocked or substituted by known growth factors or serum factors. At a concentration of 1 nM or less, AMF markedly stimulates the random and directed motility of breast cancer cells but fails to induce motility in leukocytes. Following transfection with the activated ras oncogene, AMF and its receptor are enhanced more that 100 fold in certain cells. AMF was used to address the question as to whether human A2058 melanoma cells use different matrix receptors to migrate on different substrates. AMF stimulates tumor cell motility on plastic as well As type IV collagen, type I collagen, laminin, and fibronectin. Anti-laminin and anti-laminin receptor antibodies inhibit AMF-induced migration on laminin but not fibrinectin. In contrast, RGD but not RGE abolished AMF-induced migration on fibronectin but not laminin. THus, a tumor cell can simultaneously posses different matrix receptors and use the appropriate receptor for the available matrix substrate.

Q 025 THE NEAR-DIPLOID CELLS OF ASTROCYTOMAS AND MALIGNANT GLIOMAS: THEIR CHEMORESISTANCE AND IN VITRO BEHAVIOR. Joan R. Shapiro, Anwar N. Mohamed, Salah A.D. Ebrahim, Bipin Mehta and William R. Shapiro. Memorial Sloan-Kettering, New York, N.Y. 10021.
Samples from 5 freshly resected astrocytomas (AS), 6 anaplastic astrocytomas (AA) and 10 glioblastomas multiforme (GBM) were dissociated into pools of single cells that were aliquoted for cell culture, cytogenetic analysis and colony forming assay (CFA) after BCNU treatment. Five tumors had multiple samples obtained from different regions. The AS were primarily near-diploid (2n) in chromosome number (35-57 chromosomes/metaphase), and each had a subpopulation of cells containing 45 chromosomes missing only a single sex chromosome. Less frequently represented subpopulations included cells with 46 normal G- or Q-banded chromosomes, 44 chromosomes missing chromosomes 22 and the X, 45 chromosomes monosomic for chromosomes 22 and the X and trisomic for 7, and 92 chromosomes with 4 homologues for each chromosome number. Four of 6 AA were primarily near-diploid in chromosome number and 2 contained mixtures of near-diploid along with triploid (3n), tetraploid (4n) and pentaploid (5n) populations. Of the GBM, 6 of 10 were near-diploid, the other 4 containing cells with 2n-8n chromosome numbers; karyotypes from 3n-8n cells had extensive numerical aberrations. Subpopulations identifiable in AS were also present in AA and GBM, but in proportions 3-20 fold higher. Marker chromosomes were similarly increased in representation. No specific marker chromosome was common to all tumors, although chromosomes 7 and 20 were over-represented and chromosomes 22 and the X were under-represented. In the multi-regional study, non-adjacent regions in 3 of 5 tumors contained foci of cells that were karyotypically different. Following treatment with BCNU, only tumors in which the majority of cells contained chromosome numbers greater than 3n were sensitive; all tumors that were primarily diploid were resistant to drug doses less than 15 μg/ml. Contained within the BCNU-sensitive tumors were resistant cell populations; these cells were near-diploid and differed from most of the cells in the tumor in that they had an over-representation of chromosome 22. Foci of such cells were also present in some of the samples from the multi-regional study. Crude extract and conditioned medium prepared from these BCNU-resistant cells stimulated ^{3}H-thymidine uptake in PDGF receptor-positive cells (inhibited by anti-PDGF antibody), and competed with iodinated PDGF for receptor binding, suggesting that such cells produce a PDGF-like factor to which they respond. Three of the GBM recurred in the patients after treatment. Cytogenetic analysis of these recurrent tumors demonstrated cells with 2 or more copies of chromosome 22 and 4-7 copies of chromosome 7. Conditioned medium from these cells stimulated thymidine uptake; receptor studies with iodinated PDGF are in progress. These studies suggest that some human gliomas contain subpopulations of BCNU-resistant autocrine cells that produce a PDGF-like factor, and that these cells repopulate the tumor after BCNU treatment.

Q 026 BREAST CANCER AT THE CELLULAR LEVEL, Helene S. Smith[1], Christopher Benz[2], Edison Liu[2], Britt-Marie Ljung[2], Abla Creasey[3], Charles Dollbaum[1], Adeline J. Hackett[1]
[1]Peralta Cancer Research Institute, Oakland, CA 94609, [2]University of California, S.F., CA, 94143, [3]Cetus Corp, Emeryville, CA 94608.
To relate recent advances in molecular and cell biology to human breast cancer, we utilized a short-term culture system that allows extensive proliferation of both normal and malignant human mammary cells. Cells cultured from primary breast cancers were compared to nonmalignant mammary epithelial cells to study early stages of malignancy. The tumor cells differed from nonmalignant cells by a number of criteria including invasiveness and sensitivity to tumor necrosis factor. Despite these differences, rapidly proliferating normal and malignant cells had similar, high levels of expression of the oncogenes, c-Ha-ras and c-myc. These observations suggest that oncogene overexpression by breast cancers may relate to proliferative state rather than represent an underlying difference between normal and malignant breast epithelium.

To study later stages in malignant progression, primary breast cancers were compared to metastatic lesions. Cells cultured from primary lesions were mostly diploid while those cultured from late stages in malignant progression were uniformly aneuploid. These results are best explained by Nowell's hypothesis that diploid neoplastic cells generate nonviable cells with grossly abnormal chromosomal contents. Only occasionally does this process generate a viable aneuploid cell with growth advantage allowing it to selectively populate the original tumor or subsequent metastases. We hypothesize that this evolving malignant process is quite slow in breast cancer so that the original diploid cells, the nonviable and viable aneuploid populations all coexist in the primary lesion. This model makes the following predictions all of which were found to be true: 1) many aneuploid cells will be nonviable; 2) aneuploidy will be associated with malignant progression; 3) metastases will be highly variable because they arise from multiple random genetic changes; and 4) diploid cells will be found in the primary tumors. The relative proportion of viable aneuploid and diploid tumor cells in vivo is unclear since the predominantly diploid population observed after culture may be the result of selective proliferation. These diploid tumor cells which lack many irrelevant changes generated in the metastatic cells by chromosome instability may provide important insights into fundamental differences between normal and malignant mammary epithelium.
Supported by grants from ACS (PDT-282, CH235), NCI (CA38739, CA36773) and Cetus Corp.

Liver Metastasis

Q 027 REGULATION OF GROWTH IN COLON CARCINOMA, M. Brattain, N. Hoosein, D. Boyd, K. Mulder, A. Levine, L. Watkins, S. Chakrabarty, K. Matthews and D. Brattain, Bristol-Baylor Laboratory, Baylor College of Medicine, Houston, TX 77030

The development and characterization of a large bank of colon carcinomas showing heterogeneous biological properties is described with an emphasis on the growth regulatory controls expressed by the various sub-types of cells. The establishment and biological characterization of the bank has been previously described (1). Lines in the bank were classified on the basis of in vitro and in vivo growth properties. Group I lines showed a lack of differentiation and a high degree of differentiation when grown as xenografts. Group II lines had intermediate characteristics relative to these extremes.

In order to compare both autocrine and exogenous requirements in these subclassifications, cell lines were adapted to growth in serum free, chemically defined medium and the ability of individual stimulatory growth factors (IGF's, insulin, transferrin, EGF) and combinations of growth factors to support growth were determined. Group I cell lines showed a high degree of autonomy with regard to exogenous growth factor requirements and were capable of maximal growth when supplemented with transferrin or transferrin + insulin. Group III cell lines, on the other hand, required a combination of transferrin, insulin + EGF for maximal growth. Differences in the EGF responsiveness of the two sub-types of cells were reflected by 3-10 fold higher receptor numbers in Group III lines relative to Group I lines. Conversely, Group I lines showed higher secretion of TGF than Group III lines.

The inhibitory activity of several polypeptides, some of which are produced endogenously, was characterized in the bank. Of particular interest was TGFβ which inhibited some Group III lines, but had no effect on the growth of Group I cells. Inhibition was accompanied by increased expression of fibronectin and decreased expression of c-myc. Some lines which were not inhibited by the polypeptide did respond in other ways (e.g.increased secretion of carcinoembryonic antigen or mucin). The effects of TGFβ in this system were quite similar to those of the well-known differentiation inducers retinoic acid and N,N-dimethylformamide.

1. Cancer Metastasis Reviews,3,177,1984.

Q 028 EXPERIMENTAL NUDE MICE MODEL TO INVESTIGATE HUMAN COLORECTAL LIVER METASTASES, R. GIAVAZZI, Mario Negri Institute for Pharmacological Research, Via Gavazzeni 11, 24100 Bergamo - Italy.

Since the most common site of colon carcinoma metastases is the liver we wished to investigate the capacity of human colon-derived tumor cells to produce metastases in the liver of athymic nude mice. Human colorectal carcinomas, originated from primary neoplasms and/or hepatic metastases of different patients, were enzymatically dissociated and single cell suspensions injected into different sites of nude mice. Tumor cells transplanted into the subcutis or muscularis of nude mice produced only local tumors without visceral metastases. On the contrary mice injected intrasplenically (i.s.) with colonic derived tumor cells developed tumors in the spleen and in the liver. The ability to produce liver metastases in nude mice could distinguish tumor cells with different malignant potential: the most metastatic being tumor cells originated from hepatic metastases of the patient.

Studies with [^{125}I]IdUrd-labelled colonic tumor cells suggested that tumor cells reach the liver shortly after their injection in the spleen; moreover nude mouse splenectomy, following the i.s. injection, did not significantly influence the capacity of tumor cells to grow in the liver. This suggests that the production of tumor in the liver of nude mice is determined by both the ability of tumor cells to reach the liver, and the ability of colonic tumor cells to proliferate in the liver environment.

Human colon tumors were maintained as continuous cell lines in the nude mouse: their histological and cytological characteristics, tumorigenic and metastatic potential were, in general, maintained at different passages. Isoenzyme and karyotype analysis ascertained the human origin of the tumors growing in the nude mice.

The use of this experimental model with regard to the therapy of colon tumor liver metastases will be addressed.

Q 029 ADHESION MECHANISMS IN LIVER METASTASIS FORMATION, Ed Roos, Folkert F. Roossien, Otto P. Middelkoop, John G. Collard and Geertje La Rivière, Division of Cell Biology, The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX Amsterdam, The Netherlands.
Invasion of liver by metastasizing tumor cells takes place in small vessels where no basement membrane is present. Thus, the tumor cells interact only with liver cells: sinusoidal endothelial cells and hepatocytes. These cells were isolated and the interaction with tumor cells studied in vitro. Based on the effect of polyclonal and monoclonal antibodies, toxins and drugs, we have previously concluded that the invasion mechanism of carcinoma cells was quite different from that of lymphoma cells.
Presently we have studied lymphoma invasion in more detail. We observed that normal, non-tumorigenic, activated T-lymphocytes invade hepatocyte monolayers similarly as highly metastatic lymphoma cells. This suggested that the lymphoma cells were highly invasive due to activation of their inherent invasion machinery. This notion was supported by our observation that fusion of activated normal T-cells with non-invasive and non-metastatic lymphoma cells yielded highly invasive T-cell hybridoma cells which were also highly metastatic.
The similarity of invasion mechanisms suggested that molecules involved in interactions between normal lymphocytes and other cells and tissues might play a role in liver invasion. So far, this was found to be true for LFA-1 and for a pertussis toxin substrate, i.e. an as yet unidentified signal transducing G-protein. LFA-1 is a cell surface protein involved in adhesion between leucocytes. Anti-LFA-1 antibodies were found to inhibit adhesion of lymphoma and T-cell hybridoma cells to hepatocyte cultures and consequently to reduce invasion into the monolayers. Control antibodies, directed against T200 and Thy.1, of the same isotype, at least similar affinity and similar binding levels, did not inhibit. Comparison of binding level and effect on adhesion at different antibody concentrations suggested that the antibody did not act by simple blocking of LFA-1 function. Alternative explanations will be discussed.
Pertussis toxin interferes with invasion of normal lymphocytes into tissues. We found that toxin pretreatment of lymphoma cells reduced adhesion, and consequently invasion, in hepatocyte cultures. When cells were pretreated with a high concentration, the toxin effect persisted for at least three days, despite an almost hundred fold increase in cell number.
Preliminary results in vivo indicate, surprisingly, that the toxin affects metastasis formation in the liver, but not elsewhere. This suggests that the invasion mechanism is different in liver as compared to other organs.

Future Perspectives for Therapy of Metastasis

Q 030 BIOLOGICAL APPROACHES FOR TREATMENT OF METASTASES, Isaiah J. Fidler, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, 1515 Holcombe Boulevard, Houston, Texas 77030.
The uncontrolled growth of metastases which are resistant to conventional therapies is a major cause of death from cancer. Recent data from our laboratory and many others indicate that metastases can arise from the nonrandom spread of specialized malignant cells that preexist within a primary neoplasm, that metastases can be clonal in their origin, that different metastases can originate from different progenitor cells, and that, in general, metastatic cells are less stable (genetically and epigenetically) as compared with benign nonmetastatic cells. These data provide an explanation for the clinical observation that multiple metastases exhibit heterogeneous sensitivities to therapeutic modalities. These findings imply that the successful therapy of disseminated metastases will have to circumvent the problems of neoplastic heterogeneity and the development of resistant to therapy by tumor cells.
Appropriately activated macrophages can fulfill these demanding criteria. Macrophages can be activated to become tumoricidal by interaction with phospholipid vesicles (liposomes) containing various immunomodulators, such as lymphokines, bacterial products and synthetic molecules. Tumoricidal macrophages can recognize and destroy neoplastic cells in vitro and in vivo, while leaving non-neoplastic cells unharmed. Intravenously administered liposomes are cleared from the circulation by phagocytic cells. The endocytosis of liposomes containing immunomodulators results in generating cytotoxic properties in macrophages in situ. This process is independent of the thymus and can be achieved in mice without functional T-cells such as athymic nude mice or mice immunosuppressed by Cyclosporin-A. The multiple administrations of liposomes containing immunomodulators have produced eradication of cancer metastases in several rodent-tumor systems.
Macrophage destruction of cancer metastases is limited by the ratio of effector to target cells. Thus, destruction of small metastases is effective, but once metastases exceed a certain number of cells, therapeutic efficacy is diminished. The ability of tumoricidal macrophages to distinguish neoplastic from bystander non-neoplastic cells presents an attractive possibility for treatment of the few tumor cells which escape destruction by conventional therapeutics. For this reason, we have been investigating various methods to reduce the tumor burden in metastases by modalities such as chemotherapy or radiotherapy. The results of these investigations suggest that the systemic activation of macrophages can indeed provide an effective modality for treatment of metastases.

Q 031 THE EFFECT OF LOCAL CONTROL OF THE PRIMARY TUMOR ON SUBSEQUENT METASTATIC SPREAD IN HUMAN CANCERS, Zvi Fuks,
Department of Radiation Oncology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021. Observation with several experimental tumors indicated that the excision of the primary tumor significantly increased the incidence and growth rate of distant metastases. The basic assumption in these studies was that the primary tumor exerts a suppressing effect on metastatic growth. The clinical experience in the human failed to show a similar phenomenon. Conversely there is extensive evidence with various tumors clearly demonstrating that higher local control rates of the primary tumor result in lower rates of distant metastases and higher rates of cured patients. Even in advanced stages, or when the tumor fails locally after radiation therapy, salvage aggressive surgery results in significant rates of long term survivors. Furthermore, the observed incidence of distant metastases is higher among local failure patients that among local control patients. Data will be presented to demonstrate the decreased distant metastase incidence and improved long term survival associated with more effective local therapy in tumors of the head and neck, breast, urinary bladder, prostate, ovary, and the rectum. In large cell carcinoma of the lung, the reduction of local failure by aggressive surgery and radiotherapy did not decrease the rates of distant metastases and mortality, nor did it increase it. Possible explanations for the differences observed between human tumors and the experimental tumor models in animals will be discussed.

Genetics and Epigenetics

Q 100 SIMULTANEOUS TRANSFER OF TUMORIGENIC AND METASTATIC PHENOTYPES BY TRANSFECTION WITH GENOMIC DNA FROM A HUMAN CUTANEOUS SQUAMOUS CELL CARCINOMA. Honnavara N. Ananthaswamy, Janet E. Price, Leonard H. Goldberg and C. Elise Straka, The Univ. of Texas M. D. Anderson Hosp. and Tumor Inst. and Baylor College of Medicine, Houston, TX 77030.
High molecular weight DNAs prepared from several primary human skin cancers were assayed for their ability to transform NIH 3T3 cells. A cotransfection protocol using pSV2-neo DNA, which confers resistance to the antibiotic G418, was employed to select cells that had taken up foreign DNA. About 2 wks after transfection, G418-resistant colonies from each dish were pooled and injected s.c. into athymic nude mice. Tumors grew in mice from cells transfected with DNA from 4 of 6 human skin cancers within 3-4 wks after injection. In contrast, NIH 3T3 cells transfected with pSV2-neo DNA alone or cotransfected with human placental DNA did not produce tumors within this period. Surprisingly, cells transfected with DNA from one human skin cancer not only induced s.c. tumors at the site of injection, but also metastasized spontaneously to the lungs in 100% of mice injected. All cell lines established from tumor and metastases were found to be resistant to G418. In addition, a majority of the tumors and metastases recovered from nude mice also contained human DNA sequences. These results, although preliminary, suggest that at least in one human skin cancer, tumorigenic and metastatic phenotypes may be controlled either by a single gene or by separate but closely linked genes. Alternatively, unlinked genes controlling the two phenotypes might have undergone recombination, possibly by cointegration during the course of transfection.

Q 101 BIOLOGIC CHARACTERISTICS OF NON-METASTATIC CELLS ISOLATED FROM THE MURINE K-1735 MELANOMA, Sharon Lea Aukerman, *Adriana Albini, *George R. Martin, and Isaiah J. Fidler. The University of Texas M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030 and *Laboratory for Developmental Biology and Anomalies, National Institute of Dental Research, NIH, Bethesda, MD 20205.
Cloned cell lines isolated from the K-1735 murine melanoma vary in their metastatic potentials. Several of these clones rarely metastasize and are presently under study to determine the reasons for their lack of metastatic ability. The metastatic potential of these cell lines was compared in syngeneic mice and in athymic nude mice to identify those cell lines unable to metastasize in normal syngeneic hosts due to their high antigenic phenotype. *In vivo* distribution studies of radiolabeled cells and *in vitro* invasion analysis were performed on those cell lines which were non-metastatic in any recipient mice to determine whether the lack of metastatic capacity was due to cell deficiency in: invasion, ability to aggregate, arrest in a capillary bed, invasion of basement membrane, or the ability to grow in distant organ parenchyma. Any one of these deficiencies alone or in combination could then account for the failure of cells to produce metastases. The detailed characterization of non-metastatic cell lines and the identification of their specific deficiency (ies) is a mandatory prerequisite for the use of cell lines as recipients for DNA transfection studies aimed at studying the genetic control of the nonmetastatic phenotype. Such DNA transfection studies are currently in progress now.

Q 102 DIFFERENT TYPE IV COLLAGENOLTIC RESPONSE IN NORMAL AND MALIGNANT CELLS, EXPOSED TO A TUMOR PROMOTER, Marina Ballin, Unnur P. Thorgeirsson, and Lance A. Liotta, Laboratory of Pathology, NCI, NIH, Bethesda, Md 20892. The effect of the phorbol ester tumor-promoter 12-O-tetradecanoylphorbol 13-acetate (TPA) on type IV collagen degrading activity was studied in cultured normal and malignant cells. Primary cultures of normal human adult fibroblasts (MRCS) and fetal fibroblasts (IMPR.90) at different stages of confluency were exposed to 10^{-9}-10^{-6}M, TPA for 48 hours. No change in the type IV collagenolytic activity was noted in supernatants of the two primary fibroblast cultures exposed to TPA. However, immortalized nontumorigenic mouse fibroblasts (NIH/3T3) expressed up to 300% increase in type IV collagenolytic activity, when compared to untreated cells. Similary, 500% increased in the collagenase activity was detected in supernatants from a TPA treated human metastatic melanoma (A2058) line and a fibrosarcoma (HT 1080) line. The highest collagenase activity was obtained in the presence of 5×10^{-8}M TPA. These results show that TPA stimulates type IV collagenalytic activity in immortalized fibroblasts and tumor cells, but not in primary fibroblast cultures. To study if the induction of type IV collagenase in TPA-induced cells occurs at a transcriptional level we have isolated mRNA from the A2058 melanoma cells with and without exposure to TPA. *In vitro* translation products of the mRNA from the TPA-induced cells revealed an increase in proteins corresponding in molecular weights to the mammalian and type IV collagenase. Isolation of mTNA from the normal and immortalized fibroblasts is in progress. Identification of the type IV collagenase in the translation produced will be made by immunoprecipitation with IgG from a polyclonal antibody to human tumor type IV collagenase.

Q 103 ENDOTHELIAL CELL SURFACE GLYCOPROTEINS ASSOCIATED WITH PREFERENTIAL ADHESION OF METASTATIC MURINE RAW117 LARGE CELL LYMPHOMA TO ORGAN-DERIVED MICROVESSEL ENDOTHELIAL CELLS. Paula N. Belloni, Robert J. Tressler and Garth L. Nicolson, Dept. of Tumor Biology, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030.
During blood-borne metastasis tumor cells must adhere to small vessel endothelium, often at specific organ sites. We have developed an *in vitro* adhesion assay to study tumor cell-endothelial cell interactions. The parental murine large cell lymphoma line RAW117-P of low metastatic potential and two sublines selected *in vivo* for increased liver (RAW117-H10) and lung (RAW117-L17) colonization were tested for differences in adhesion to murine hepatic sinusoidal (HSE) and lung microvessel (LE) and bovine aortic (BAE) endothelial cell monolayers. Radiolabeled tumor cells were incubated with the monolayers + shear, and adhesion was determined. Adhesion of H10 and L17 cells to HSE was 2-3 times higher than for P cells, but only lung-colonizing L17 cells bound preferentially to LE. These results were consistant with metastasis data. When assayed on BAE or collagens, differences were not found, and the numbers of adherent cells were lower than for HSE or LE. Endothelial cell glycoproteins that bind to tumor cells were isolated by adsorbing labeled cell lysates onto fixed tumor cells, and then analyzed by SDS-PAGE and Western lectin staining. Different amounts of glycoproteins from HSE, LE and BAE were bound to the different tumor sublines, suggesting that specific endothelial glycoproteins are important in tumor cell recognition. Supported by NIH grant RO1-CA29571 to G. L. Nicolson.

Q 104 EVIDENCE OF GENE AMPLIFICATION AS A MECHANISM FOR TUMOR CELL INVASION, Sandra J. Bevacqua, Christopher W. Greeff, and Mary J.C. Hendrix, Departments of Molecular and Cellular Biology, Microbiology and Immunology, and Anatomy, University of Arizona, Tucson AZ 85724. In order to study the process by which human melanoma cells achieve invasion of basement membranes, a modification of the Membrane Invasion Culture System (MICS, Gehlsen, et al., 1984), called Mega MICS, was developed to allow the in vitro collection of large populations of human melanoma cell that had simultaneously invaded acellular human amniotic membranes. A significant increase in the number of double minute chromosomes (DMs) was observed in metaphase nuclei of A375P human melanoma cells which had passed through 2 amniotic membranes (A375P-2) over that of control cells. Eighteen percent of the first monolayer of A375P-2 cells contained 1-89 DMs/cell, whereas 3% of the control A375P cells contained 1-4 DMs/cell. There was a rapid loss of DMs in A375P-2 cells as a function of passage number. After 25 days in tissue culture, the incidence of DMs had essentially dropped below the control range. This indicates that an unstable gene amplification event may be part of the process by which melanoma cells execute invasion through basement membranes.
Research supported by NIH IROI CA42475 to M.J.C.H. and NIH Training Grant to S.J.B.

Q 105 TUMOR PROMOTOR INDUCED ALTERATIONS IN TUMOR RESISTANCE TO NATURAL DEFENCE, Donna A. Chow and Paul A. Sandstrom, University of Manitoba, Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada, R3E OV9.
Growth of established murine tumor lines in media containing the phorbol ester tumor promotor 12-O-tetradecanoylphorbol-13-acetate (TPA), was associated with reductions in in vitro and in vivo parameters of natural resistance. L5178Y-F9 cells exposed to 100 ng TPA/ml for 2 days exhibited reductions in sensitivity to complement mediated lysis of natural antibodies (NAb) and to hypotonic lysis, the end point in many mechanisms of cell death. In addition to changes in these parameters, the natural killer (NK) cell sensitive SL2-5 lymphoma was less NK sensitive after 2 days growth in 2 ug TPA/ml. Initial experiments show that the TPA treated cells bound less NAb measured through fluorescence detection at 37°C. The tumor frequency of threshold subcutaneous inocula of the TPA treated tumors was increased in syngeneic DBA/2 mice revealing a correlation between the reductions in the cellular characteristics assayed in vitro and susceptibiltiy to natural resistance in vivo for the TPA treated tumors. Upon return to tissue culture in the absence of TPA a reversal was seen in the in vitro parameters indicating the requirment for TPA to maintain the natural defence resistant phenotype. These data suggest that reversable TPA induced reductions in sensitivity to mediators of natural resistance contribute to tumor survival in vivo increasing the probability that the tumor will progress to a more malignant phenotype. Supported by the MRC of Canada.

Q 106 TRANSFECTION OF LM FIBROBLASTS WITH DNA FROM MOUSE MELANOMA CELLS: SELECTION OF VIABLE TUMOR-ANTIGEN-POSITIVE COLONIES BY ERYTHROCYTE-ROSETTING AND THE INDUCTION OF AN ANTI TUMOR RESPONSE WITH ANTIGEN-POSITIVE TRANSFECTED CELLS. E.P. Cohen, Y. Kim, R. Slomski and K.L. Hagen, University of Illinois at Chicago, Chicago, IL 60612.

LM cells, a mouse fibroblast cell line, (a thymidine kinase-deficient variant was used to facilitate selection), were co-transfected by electroporation with DNA from B16 cells, a mouse melanoma, and pTK, a plasmid carrying the thymidine kinase gene. Viable, antigen-positive colonies of transfectants expressing antigens cross-reactive with those formed by B16 cells were identified by erythrocyte rosetting in situ, using an antiserum raised in histocompatible C57Bl mice injected with (x-irradiated) B16 cells. Mouse anti B16 serum was reacted with HAT-resistant transfected LM cells, followed by human RBC's coated with anti-mouse immunoglobulin. Colonies with adherent RBC's were selectively removed with EDTA and expanded in culture for later analysis. Fourteen antigen-positive colonies were identified among approximately 6000 colonies of LM cells. Nine were analyzed by flow cytometry. The proportion of positively-staining cells in each isolate ranged from 17 to 82 percent ($\bar{x}$=40); antigen-negative colonies failed to stain. Antigen-positive (and antigen-negative) transfectants were used to immunize C57Bl mice. One month after immunization, animals injected with antigen-positive (but not antigen-negative) transfected cells exhibited evidence of cellular immunity toward B16 cells.

Q 107 EXPRESSION OF THE ERV3 PROVIRUS IN NORMAL AND MALIGNANT HUMAN REPRODUCTIVE TISSUES, Maurice Cohen[1], Erik Larsson[2], and Nobuyuki Kato[1]. 1. BRI-Basic Research Program, NCI- Frederick Cancer Research Facility, Frederick, MD 21701. 2. University of Uppsala, Uppsala, Sweden.

Three poly (A) + RNAs of 9, 7.3, and 3.5 kb of the single copy human provirus, ERV3, are abundant in the chorion of normal first trimester and term human placenta, representing 0.1% of the total mRNA. Northern blot hybridization and S1 nuclease analyses have revealed that the three RNAs are all spliced, subgenomic proviral transcripts. Whereas the 3.5 kb transcript terminates in the ERV3 3' LTR, the 9 and 7.3 kb transcripts extend through the 3' LTR and are spliced again about 370 bp downstream of the LTR in the 3' flanking region. No full length ERV3 transcript could be detected. This represents the first known example of endogenous proviral transcripts containing genomic sequences. These results suggest that when the ERV3 provirus integrated in a human ancestor, it may have integrated within or adjacent to a cellular gene. In addition to its high expression in normal human chorion, ERV3 is also expressed in many other normal and malignant human tissues and cells, but at a significantly lower level. In Northern blot analyses, an ERV3 env-specific probe detected ERV3 transcripts in several female reproductive tumor cell lines including endometrial and cervical carcinomas. In contrast, the probe failed to detect any ERV3 transcripts in 3 out of 3 choriocarcinoma cell lines although these RNAs are particularly abundant in normal chorion. The biological significance of these results will be discussed.

Research sponsored by NCI under contract NO. NO1-CO-23909 with Bionetics Research, Inc.

Q 108 C-HA-RAS AND GENES ON HUMAN CHROMOSOME 7 INDUCE INVASIVE AND METASTATIC POTENTIAL. J.G. Collard[1], J.F. Schijven[1], M.v.d.Poll[1], A. Scheffer[1], E. Roos[1], J.J.M. van Dongen[2] and A.H.M. Geurts van Kessel[2]. The Netherlands Cancer Institute[1], Amsterdam; Erasmus University[2], Rotterdam, The Netherlands.

Non-invasive, non-metastatic BW5147 T-lymphoma cells transfected with the human c-Ha-ras oncogene acquire invasive properties and metastatic potential. The induced level of both invasiveness, as assessed in hepatocyte cultures, and metastatic potential, assessed after tail vein injection into syngeneic AKR mice, is dependent on the expression of the ras gene. Transfectants with a high copy number and high expression of the ras gene showed the highest invasiveness and produced widespread metastasis in all mice tested. Cells with low levels of ras expression were less invasive and formed metastases in a few mice only, limited to a few organs. Control transfectants without the ras gene were non-invasive and non-metastatic.

Mouse T-cell hybrids prepared by fusion of the same BW cells and activated normal mouse T-cells also became invasive and metastatic, suggesting that these properties were derived from the parental normal T-cells. Chromosome analysis of interspecies mouse-human T-cell hybrids in vitro selected for invasiveness and low numbers of human chromosomes, revealed that the acquisition of invasiveness of the hybrids depended completely on the presence of human chromosome 7. These invasive interspecies hybrids produced metastases after tail vein injection in nude mice in contrast to non-invasive hybrids lacking human chromosome 7. We conclude that in addition to the activated ras oncogene, (a) gene(s) expressed in activated T-cells and located on human chromosome 7 are also able to induce invasiveness and metastatic potential in T-lymphoma cells.

Q 109 HA-RAS ENHANCEMENT OF PROGRESSION IN AD5-TRANSFORMED RODENT CELLS. G.J. Duigou[1], S.G. Zimmer[2], and P.B. Fisher[1]. Columbia Univ.[1], N.Y., N.Y., 10032 and Univ. of Kentucky[2], Lexington, KY. The Molecular basis of tumor cell progression is not known. We have previously characterized progressed adenovirus-transformed Sprague Dawley rat embryo clones isolated from tumors induced in nude mice (E11-NMT) stably differing from their parental cells (E11) by enhanced expression of transformation-associated traits that were not regulated by altered expression of integrated Ad5 DNA or the endogenous Ha-ras gene. Cells displaying a progressed phenotype have also been isolated from E11 cells following selection in agar and progressed cells can be reverted to the E11 stage of transformation by 5-azacytidine. To determine if progression of E11 cells could be induced by the introduction of exogenous oncogenes, an activated human Ha-ras (T24) oncogene was introduced into E11 cells by cotransfection with pSV2-neo and G418-resistant colonies were isolated. Six of the transfected clones exhibited increased growth in 0.4% agar (12-53%) compared to E11 cells (0.5-2%), transfected clones R5 (1%) and R14 (2.6%). Southern blot analyses of DNA from these cell lines revealed no gross rearrangements of integrated Ad5 DNA and a positive correlation between increased growth in agar and multiple copies of exogenous ras DNA. The ras oncogene, when expressed in an immortal rat embryo cell line (CREF), was able to confer a tumorigenic and metastatic phenotype to nontumorigenic wild-type Ad5-transformed CREF cells. These model systems should prove valuable in analyzing the interaction between adenovirus transforming genes and cellular oncogenes in regulating expression and progression of transformed state.

Q 110 DIRECT REGULATION OF THE METASTATIC PHENOTYPE IN ESTABLISHED RODENT FIBROBLASTS BY NORMAL AND ACTIVATED H-ras, Sean E. Egan, Lenka Jarolim, Jim A. Wright, Arnold H. Greenberg, Manitoba Institute of Cell Biology, 100 Olivia St., Wpg., Man., Canada, R3E 0V9.

Experiments have been undertaken to establish the relationship between ras expression and metastatic potential in C3H-10T½ and NIH-3T3 cells. The metastatic potential (experimental and spontaneous), tumorigenicity and soft agar cloning ability of five ras transfected 10T½ cell lines all followed the same relationship and correlated with ras RNA levels suggesting the coregulation of these phenotypes by ras. In addition, preinduction of MMTV LTR promoted v-H-ras in NIH-3T3 line 433 by dexamethasone significantly enhanced the metastatic potential of this cell line. Contradictory reports have appeared in the literature dealing with the ability of the c-H-ras proto-oncogene to convert NIH-3T3 to metastatic tumors. In order to resolve this question we have used several previously characterized cell lines (Nature 297:479, 1982, Mol. Cell. Biol. 5:2836, 1985) and found that the p21 level correlates with metastatic potential. At least 30 fold overexpression of normal p21 appears to be necessary for significant ras mediated metastatic activity suggesting that previous negative findings may have been due to examining cell lines with insufficient ras expression. DNA isolated from 6 lung tumors in 3 mice were unable to induce focus formation in 3T3 cells. Therefore metastases did not result from spontaneous activation of c-H-ras sequences during transfection or in vivo growth. Taken together, these results provide strong evidence for direct regulation of the metastatic phenotype by both normal and activated ras genes in NIH-3T3 and C3H-10T½ cells. (Supported by the N.C.I.C. and M.H.R.C.)

Q 111 ISOLATION OF A cDNA REPRESENTING A METASTASIS-ASSOCIATED mRNA IN COLORECTAL CANCER, Paul Elvin[1], Ian B. Kerr[2], George D. Birnie[1] and Colin S. McArdle[2], Beatson Institute for Cancer Research[1] and Royal Infirmary[2], Glasgow, Scotland.

The presence or absence of 'occult' metastatic disease at the time of diagnosis has been shown to be a critical prognostic factor for patients with colorectal cancer. Although many features of tumours have been studied in relation to the metastatic phenotype, there are no clinically applicable markers for the unequivocal detection of metastases or for the determination of the metastatic potential of a tumour.

Phenotypic changes associated with metastasis are expected to be reflected in subtle changes in the activity of many gene loci. To investigate changes in gene expression arising through the development of colorectal metastases, we have differentially screened a cDNA library from a liver metastasis with cDNA probes derived from the total poly(A)$^+$ RNAs of primary colorectal tumours, liver metastases of colorectal tumours, and normal colonic mucosae. We have identified a cDNA clone, pLM59, of approximately 300bp homologous to a 0.8 kb RNA that is of four-fold increased abundance in metastases relative to primary tumours, and six-fold increased abundance in metastases relative to normal mucosae. The homologous RNA is also poorly represented in the total RNA of normal human liver.

No sequences homologous to the cDNA of pLM59 were found in the GenBank sequence data bank. Thus this sequence represents a hitherto uncharacterised gene associated with the development of metastases in colorectal tumours. As such the clone may be of clinical importance in the diagnosis of colorectal tumours and their metastases, and may also provide an insight into the mechanisms underlying the development of metastasis.

Q 112 GENETICALLY CONTROLLED IMMUNE RESPONSE IN C57Bl/6 MICE AGAINST AN H-2 DEFICIENT CELL VARIANT OF L5178Y, Enrique Escandón, Alicia Sampieri, Veronica Yakoleff, Gabriel Nava and Guillermo Alfaro, Department of Immunology, National University of Mexico and Department of Molecular Immunology, National Cancer Institute, Mexico.

The growth of murine tumors is generally H-2 restricted when transplanted in mice of a different haplotype. However, in some cases, it is possible to isolate cell variants which are capable to grow across allogenic barriers and to overcome this restriction. We have isolated and H-2 defective cell variant (LR) from L5178Y ($H\text{-}2^d$) which grew well in ascitis form in mice of different haplotypes, such as $H\text{-}2^b$, $H\text{-}2^d$ and $H\text{-}2^k$. Of the mouse strains tested, C57Bl/6 alone rejected the tumor. Specific immunity to LR was demonstrated since, in subsequent challenges of these mice with either LR or EL4 ($H\text{-}2^b$), LR did not grow, whereas EL4 grew and killed the mice. The immune response in C57Bl/6 was mediated by specific antibodies and protected the animals for over one hundred days after the tumor had been rejected. Also, when F1 mice (C57Bl/6 X BALB.b) were used, it became apparent that the capacity to reject the tumor was a dominant trait; however, since in most of the animals the tumor recurred, it was concluded that the immune response in F1 mice was different to that of C57Bl/6 due to its modulation by the genetic background of the other animal used in the cross.

Q 113 RNA TUMOR VIRUS CONTROL OF CLASS I MAJOR HISTOCOMPATIBILITY ANTIGEN EXPRESSION. Douglas V. Faller, Susan P. Perrine and Margaret Offermann, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02115

Alterations in expression of MHC antigens on tumor cells clearly correlate with the metastatic potential of those cells, presumably secondary to resulting changes in their susceptibility to immune recognition and destruction. Murine leukemia viruses (MuLV) and sarcoma viruses (MSV) exert regulatory effects on class I genes of the MHC locus. MuLV infection results in substantial increases in cell surface expression of all three class I MHC antigens, whereas coinfection with a transforming MSV is able to override this MuLV-induced up-regulation. Both of these viral effects on MHC antigen expression profoundly influence immune-mediated destruction of the infected cells, as assessed by cytotoxic T lymphocyte recognition and killing. Control of class I MHC and beta-2 microglobulin genes by MuLV, and probably by MSV, takes place at the transcriptional level, as indicated by nuclear run-off studies and steady-state mRNA analysis. MuLV controls expression of widely separated endogenous cellular genes, transfected class I MHC genes, and unintegrated chimeric genes consisting of fragments of class I MHC genes linked to a bacterial gene. These findings indicate that MuLV exerts its effects on MHC expression via a *trans* mechanism at the transcriptional level. The MuLV-responsive sequences on the MHC genes lie within 1.2 kb upstream of the initiation codon for those genes. Infection with a transforming MSV also inhibits interferon-mediated upregulation of class I MHC antigen expression, and this repression appears to act predominantly at the post-transcriptional level.

Q 114 RNA ENCODING A METALLOPROTEASE IS ELEVATED DURING TUMOR PROGRESSION, Joanne Finch, Lawrence E. Ostrowski, Lynn Matrisian and G. Tim Bowden, Department of Radiation Oncology, University of Arizona, Tucson, AZ (J.F., L.E.O., G.T.B.) and Department of Cell Biology, Vanderbilt University, Nashville, TN (L.M.)

We have reported that transin RNA, a 1.9 Kb RNA coding for a novel, secreted protease was overexpressed during progression of benign mouse skin papillomas to malignant squamous cell carcinomas (SSC). Recently it has been demonstrated that there is a high degree of homology between rabbit stromelysin, a secreted metalloprotease which degrades proteoglycan found in the basement membrane, and the amino acid sequence predicted from rat transin cDNA (personal communication from P. Herrlich). DNA sequencing of a mouse cDNA isolated from a λgt10 cDNA library made from a MNNG induced SSC showed greater than 85% nucleotide homology with the rat transin cDNA. Using this mouse transin cDNA as a probe, we found enhanced expression of transin mRNA in SSC induced by a protocol giving rise to metastatic tumors (repeated MNNG treatment) compared to the level found in nonmetastatic SSCs (MNNG + TPA). Southern analysis of DNA isolated from SSCs induced by repeated MNNG treatment showed evidence of either gene amplification or rearrangement in certain tumors. Phorbol ester tumor promoters but not a nonpromoting hyperplastic agent induced the transient expression of transin mRNA in normal adult epidermis. The functional role of the upregulation of the transin sequence in tumor invasion and metastasis is being investigated.

Q 115 IN VIVO METASTATIC PROGRESSION OF H-ras TRANSFORMED 10T½ IS DUE TO UNSTABLE EPIGENETIC AND ras-INDEPENDENT EVENTS, M.-C. Gingras, L. Jarolim, A.H. Greenberg, Manitoba Institute of Cell Biology, 100 Olivia St., Wpg., Man., Canada, R3E 0V9.
Experiments from this laboratory have demonstrated that the metastatic efficiency of H-ras transfected fibroblasts is closely related to the expression of the oncogene. If the ability to survive in the lung is solely related to ras expression, preferential selection of high H-ras expressing, increasingly metastatic variants would be detected during early lung growth. A 10T½ fibroblast clone transformed by the pSV2 neo plasmid containing a 6.6 kb Bam Hl T24 H-ras insert, was injected i.v. in C3H/HeN mice. Tumor cells in the lungs were recovered at different times after injection (30 min., 1,9 and 21 days) by enzymatic treatment followed by selective growth in G418, and the metastatic efficiency was tested again in C3H/HeN mice. Tumor cells recovered after 30 min. were slightly higher than in vitro controls, while those surviving for 1 and 9 days were increasingly metastatic (7.7 and 26 fold, respectively). By 21 days, however, recovered tumor was almost at day 1 levels. This instability of the metastatic cells in vivo was also observed in vitro. Northern blotting demonstrated that the increased metastatic ability was not related to the transcriptional activity of the ras gene. We conclude that while the metastatic efficiency of ras transformed lines is dependent on the expression of the ras gene, metastatic progression in vivo can be ras independent. The survival of the cells in the lung seems to be related to unstable epigenetic events which can be detected during the first days of implantation. (Supported by the N.C.I.C. and M.H.R.C.)

Q 116 IMMORTALIZATION AND TRANSFORMATION OF PRIMARY HUMAN COLON CELLS, Charles Hsu, Lisa Peterson, Michael McGrogan, Laura Silberstein and Christian Simonsen, Invitron Corp., Redwood City, California 94063

Transfection of primary human colon cells with Adeno V E1a genes yielded lines with extended lifespans in culture: some isolates have been passaged over 20 generations beyond crisis, but exhibit no other transformed characteristics. By contrast, treatment with 10 ng/ml PMA transiently induces transformed characters in bulk cultures, including rapid and disorganised growth and increased plasminogen activator (PA) release. However, very few stably tranformed foci arise, and these show no extension of lifespan in culture, reaching crisis at least as fast as control cultures. Surprisingly, the E1a-transfected cells show reduced sensitivity to PMA induction, and are not readily promoted to the fully transformed phenotype. We were also unable to obtain full transformation of these cells using E1a and activated ras genes. Treatment with SV40 DNA yielded 100 transformed foci per 10•6 cells treated. These cells exhibit reduced contact inhibition and a variety of morphologies, growth rates and PA levels. Further study is focussing on the relationship of immortalisation to transformation in these and other primary human cells.

Q 117 IDENTIFICATION OF onc F, A NOVEL HUMAN ONCOGENE WITH UNIQUE BIOLOGICAL PROPERTIES. S. Katzav, D. Martin-Zanca, & M. Barbacid. Frederick Cancer Research Facility, Basic Research Program, P.O. Box B, Frederick, MD 21701.
We have examined the existance of genes potentially involved in metastasis by testing human DNAs isolated from metastatic tumors (32) as well as from primary tumors (8) in gene transfer assays. Recipient cells, including NIH/3T3 cells transformed by the v-Ki-ras oncogene, a spontaneously transformed NIH/3T3 variant and L cells were co-transfected with these human tumor DNAs and pSV2 neo by the calcium precipitation technique. Transfected cells were selected by their resistance to G418 and inoculated in the footpath of inbred NFS mice. Following detection of tumor growth, the tumored leg was amputated and the animals kept for up to 3 months, a time at which they were examined for the presence of metastasis. No metastatic tissues carrying donor DNA (human Alu) sequences were detected. In parallel control experiments the same DNAs were transfected into NIH/3T3 cells and tested in the standard nude mouse tumorigenicity assay. Seven of these DNAs exhibited transforming genes, of which six were identified as members of the ras gene family. The seventh oncogene, derived from an esophageal carcinoma of the Linxian area (sent by Dr. R. Montesano, W.H.O.) was shown to be a novel transforming gene, designated onc F. Tumors induced by serial cycles of transfection with DNAs isolated from onc F-derived NIH/3T3 transformants consistently exhibit three Alu^+ Eco RI DNA fragments of 30, 9 and 4 kbp. NIH/3T3 cells transformed by onc F possess unique properties. They are not morphologically transformed, grow very poorly in semi-solid media, and exhibit 50 to 100 copies of stably integrated onc F DNA sequences. Undergoing molecular cloning of onc F should help to understand the nature of this novel type of human oncogene.

Q 118 CHROMOSOME CHANGES IN X-RAY AND NMU-TREATED C57BL/6J MICE AT DIFFERENT STAGES OF TUMOR DEVELOPMENT, Lydia E. McMorrow, Elizabeth W. Newcomb and Angel Pellicer, UMDNJ-School of Osteopathic Medicine, Camden NJ 08103 and NYU Medical Center, New York NY 10016. Thymocytes from irradiated and NMU-treated C57BL/6J mice have been monitored for expression of differentiation antigens and chromosome aberrations during the latency period for T-cell lymphoma development. Trisomy 15 was found in a significant number of leukemic animals whether the lymphoma was induced by irradiation (46%) or NMU-treatment (70%). Trisomy 15 could also be detected in preleukemic animals without overt disease. Of the NMU-treated animals defined as preleukemic, 62% were observed to have trisomy, while 59% of the irradiated, preleukemic animals were found to have trisomy 15. A smaller proportion of treated animals, NMU- and X-irradiated, which did not exhibit any histologic or serological signs of disease were found to have trisomy 15. In addition, a marker chromosome was observed in the irradiated animals. This marker was identified in about one-third of the animals, both leukemic and preleukemic. This marker, a large, unmatched telocentric chromosome, has not been reported previously and may be an important contributing factor in tumor progression. Our studies show that chromosome aberrations occur early, prior to the development of the preleukemic disease stage and therefore, may be an important marker of preneoplastic cells.

Q 119 DIFFERENTIAL FREQUENCY OF SPONTANEOUS FUSION BETWEEN METASTATIC AND NONMETASTATIC SUBPOPULATIONS OF A MOUSE MAMMARY TUMOR, Fred R. Miller, Michigan Cancer Foundation, Detroit MI 48201. Subpopulations 66, 66cl4 (a variant of 66 which is resistant to both thioguanine [TG^r] and ouabain [Oua^r]), 410.4, and 44FTO (TG^r, Oua^r 410.4) spontaneously metastasize. Sublines 168, 168FARO (diaminopurine resistant and Oua^r 168), 67, 68H, and 410 are nonmetastatic. The ability of these lines to spontaneously fuse in vitro was determined by coculturing $5X10^5$ cells of a drug resistant line with $5X10^5$ cells of a wild type subpopulation in non-selective media. After 48 hours, cells from this fusion mixture were plated in selective media, either HAT plus ouabain or alanosine, adenine, and ouabain, to determine the number of colony forming hybrid cells present per 10^4 cells plated. In some instances, fluorescent cytometric analysis of DNA content was used to confirm that the selected cells were fusion products. When both lines of the pair in the fusion mixture were metastatic, a significantly greater ($P<0.002$) number of hybrids were recovered (median 13 per 10^4 cells) than if one of the lines in the fusion pair was nonmetastatic (median 0.6) with the exception of line 410 which readily fused with both 66cl4 and 44FTO. Line 410 was highly metastatic when originally isolated but lost its metastatic competence after a brief time in tissue culture. Thus, it apparently lost some independent property necessary for metastatic competence. The property of these metastatic cell lines which renders then more likely to fuse may be necessary but is not sufficient to endow the cell with metastatic competence. The correlation can be made with spontaneous metastatic competence only. Both 168 and 168FARO are proficient lung colonizers following intravenous injections. CA28366

Q 120 TUMOR PROMOTERS AND CELL-CELL COMMUNICATION IN RAT MAMMARY TUMOR CELLS OF VARYING SPONTANEOUS METASTATIC POTENTIALS, Garth L. Nicolson, Kim Dulski, Thomas Lembo and James E. Trosko, Dept. of Tumor Biology, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030 and Dept. of Pediatrics and Human Development, Michigan State University, East Lansing, MI 48824.

Cell-cell communication is thought to be important in cellular proliferation, differentiation, promotion, transformation and teratogenesis. We have used rat 13762NF mammary carcinoma cells of high (MTLn3), intermediate (MTF7), and low (MTC, MTPa) spontaneous metastatic potentials to examine the relationship of cell-cell communication to epithelial cell malignancy. Using a dye transfer technique to assess intercellular communication between 13762NF cells in culture we found that in this metastatic system cell coupling is inversely associated with metastatic potential (MTC, MTPa > MTF7 > MTLn3). The highest metastatic potential cells (MTLn3) failed completely to transfer low Mr dyes between adjacient cells, while the lowest metastatic potential cells (MTC) were vertually completely coupled. Addition of 12-O-tetradecanoylphorbol-13-acetate (TPA) at various concentrations (1-100 ng/ml) to cell-cell coupled cultures of MTC cells reduced or eliminated dye transfer between cells. Upon removal of TPA, the MTC cell cultures reverted back to their coupled state. Treatment of MTC cells with TPA also modified their phenotypic stability and proliferation *in vitro*.

Supported by NIH grants RO1-CA28844 (to G.L.Nicolson) and RO1-CA21104 (to J.E.Trosko).

Tumor Progression and Metastasis

Q 121 INHIBITION OF TUMORPROGRESSION AND METASTASIS BY THE IMMUNEMODULATING AGENT UKRAIN, J.W. Nowicky, Ukrainian Anti-Cancer Institute, Laimgrubengasse 19/5, A - 1060 Vienna, Austria.
Ukrain has different immunomodulating properties like increasing the T-Helper/T-Suppressor ratio and activating NK-cells. The control functions of these regulative mechanisms on the growth of tumors and their metastases are well know. Beyond a direct influencing the course of oncological diseases by immunologically stimulating mechanisms, it has become evident in the case of solitary tumors, the tendency to form metastases during surgery decreased, when Ukrain has been applied before. Activation of NK - cells and other immunstimulating mechanisms as well as a possible unmasking of tumor specific antigens seem to be the reason for all this. The formation of metastases may also be inhibited by the fact that, in successfully treated patients, the accumulation around and within the tumor can be easily proved under UV light because of its autofluorescence, thus demonstrating the process of unmasking. Moreover, demarcation phenomena occur during radiological checks and surgical interventions, inasmuch as Ukrain induces a seperation or isolation of the tumor from the surrounding healthy tissue by encapsulation. These results could be especially observed with osteosarcomas, malign melanomas and various sarcomas.

Q 122 CELLULAR AND MOLECULAR HETEROGENEITY IN TWO RAT ASCITIC TUMOURS, Gopal Pande, Ashok Khar, Ch. Sudhakar and M.R. Das, Centre for Cellular & Molecular Biology, Uppal Road, Hyderabad 500 007, India
Cellular and molecular heterogeneity has been studied in two transplantable rat ascitic tumours, namely the Zajdela ascitic hepatoma (ZAH) and the histiocytoma AK-5. In the ZAH system we have separated, by buoyant density centrifugation, two populations of cells and characterised them by flowcytometry. Our studies show that ZAH is composed of two types of cells - the lighter L cells and the heavier H cells, which are markedly different in their division and tumourigenic potentials. While L cells cause slow growing regressive tumours the H cells grow fast as malignant tumours (Cancer Res., 46, 1673). Further studies on these cells have shown that these populations are inter convertible and grow together in the tumour. We have now shown that these cells differ in their organisation of 28S RNA genes, which leads to differences in the structure of the heavy subunit of the ribosomes and affects the translation rates in these two cells. Using the AK-5 cells we have demonstrated that this tumour of macrophage origin shows a differential expression of fibronectin on the cell surface. Those cells which have a high expression of fibronectin on the cell surface are capable of growth as solid tumours while those with less or no fibronectin on the cell surface can not grow as solid tumours but grow as ascitic tumours. The potential of cells to grow as solid tumours is abolished when the cells are pretreated with antifibronectin antibody. Our studies thus show a necessary requirement of fibronectin on these cells for growth as solid tumours.Using these two tumour lines, we have demonstrated a differential control of growth and gene expression in tumour cells which makes them as good model systems for understanding tumour progression.

Q 123 Modulation of *myc* and *abl* Activity in Human Atherosclerotic Plaque DNA-associated Nude Mouse Tumors and Primary Explants. Joan Lee Parkes, Seymour J. Garte, and Arthur Penn, Dept. Env. Med., NYU Med. Ctr., 550 1st Ave, New York, NY 10016.
We report a novel tumor system which displays *myc* and *abl* gene amplifications and translocations *in vivo* and subsequent reversions to the normal gene pattern, *ex vivo*. The tumors arise following injection of NIH3T3 cells, transfected with human coronary artery plaque (hCAP) DNA into athymic (nude) mice (Penn, et al., PNAS 83: 7951-55, 1986). Primary (1^{o}) focus cell DNA and hCAP-associated nude mouse tumor (NMT) DNA were screened with 12 oncogene probes. For 10 of these genes there was no evidence for oncogene activation in DNA from foci or NMTs. However, *myc* and *abl* were dramatically amplified and/or rearranged in all the hCAP-associated NMTs. Hind III digested, Southern blotted NMT DNA probed with the 3rd exon of human c-*myc* displayed different degrees of amplification and apparent translocations in the various tumors assayed. However, bands of ~6 and ~7 kb, not present in either NIH3T3 cell or nude mouse tissue DNAs, were found in all hCAP-associated NMT DNAs tested. All hCAP-associated NMT DNAs, digested with Hind III and hybridized to an *abl* probe, displayed a band of ~10.5 kb that is similar in size to a human *abl* band. This band was not present in Hind III digested, untransformed NIH3T3 cell DNA. All the above *myc* and *abl* alterations were reversed after introduction of tumor tissue fragments into primary culture; i.e., all 1^{o} explants displayed normal mouse *myc* and *abl* patterns. This pronounced modulation of *myc* and *abl* activity appears not to be characteristic of all NMT systems but rather to be confined to hCAP-associated NMTs and their 1^{o} explants. (Supported by NIEHS 02143 and 00260 and BRSG #S07RR5399-25).

Q 124 USE OF ANTI-SENSE RNA TO STUDY THE ROLE OF UROKINASE IN ONCOGENIC TRANSFORMATION
A.Riccio and F. Blasi.
International Institute of Genetics and Biophysics, IIGB-CNR, via Marconi 10, 80125 Naples, Italy. High levels of urokinase (u-PA) or more generally plasminogen activators have been associated with the neoplastic phenotype although no direct evidence on its role is available. It has been shown that the introduction or synthesis in a cell of an RNA whose sequence is complementary to an endogenous mRNA can suppress the expression of a specific gene. In order to define the role of u-PA in transformed phenotype we decided to specifically suppress the expression of the u-PA gene by anti-sense RNA in an MSV-transformed murine fibroblast cell line (LO cells), which produces high levels of u-PA.

Anti-sense plasmids were constructed inserting several murine u-PA cDNA fragments (ranging from 80 to 2000 bp) in the flipped orientation into a vector containing the RSV-LTR promoter and an SV40 early transcript polyadenylation signal. LO cells were either transfected with pRSV-NEO, a plasmid carrying the G-418 resistance, or cotransfected with pRSV-NEO and a u-PA anti-sense construct. Cells resistant to G-418 were selected. Single colonies were picked and expanded in the presence of G-418. Several clones either transfected with pRSV-NEO alone or cotransfected with pRSV-NEO and anti-sense plasmids were tested and the amount of u-PA in the media was quantitated by ELISA assay. Some of the anti-sense clones show reduced u-PA levels in the media associated with a change in cell and colony morphology.

Q 125 CELL SURFACE GLYCOPEPTIDES OF LUNG AND LIVER COLONIZING F9 CELLS, Dario Rusciano and Benedetto Terrana, Sclavo Research Center, Siena, Italy.
We have tested the ability of the Embryonal Carcinoma cell line F9 to colonize the different organs of the syngeneic mouse after i.v. injection. We found that the liver was the most colonized organ in the large majority of tumor-bearing animals, while the lungs were only rarely involved. When F9 cells induced to differentiate by treatment with retinoic acid and dibutyrril cyclic AMP were injected as above, we observed a dramatic shift in the organ colonized: the liver was only slightly involved, while the lungs were chiefly colonized. There is a lot of emerging evidence that suggests a role of the cell surface glycoconjugates in determining the specificity of the interactions between the tumor cell and the colonized organ. We focused our attention on the cell surface glycopeptides, and found that differentiation determines both a decrease in the synthesis of the HMW polylactosaminoglycans typical of the ECC, and an increase in the synthesis of lower m.w. glycopeptides. Moreover, a further analysis of the large polylactosaminoglycan showed that this material comprises at least three species, H1, H2 and H3, and that treatment with RA only determines a decrease in the synthesis of H3 glycopeptides. The H1 material, first described in our reports, is completely sialylated, is not the result of incomplete pronase digestion, is sensitive to the action of tunicamycin, and is susceptible to endo ß-galactosidase digestion.

Q 126 STUDIES ON THE ROLE OF HOST MAST CELLS IN TUMOR ANGIOGENESIS AND METASTASIS USING THE W/W^V MAST CELL DEFICIENT MOUSE, Jean R. Starkey and Patricia K. Crowle, Montana State University, Bozeman MT 59717.
The W/W^V mouse is almost totally deficient in mucosal and connective tissue mast cells. Conflicting reports exist concerning the role of mast cells in neoplastic disease: reports of cytotoxic activity, in vitro evidence suggesting a role in angiogenesis and evidence that mast cells could aid in tumor invasion. We examined the growth, angiogenic response and spontaneous metastasis of a line of B16BL6 melanoma cells (which were insensitive to killing by murine mast cells) in W/W^V and control littermate mice. We inoculated 10^5 tumor cells subcutaneously into the external ears of 25 W/W^V and 25 control mice. The tumor latent periods, incidence, growth rates and the incidence of spread to the draining lymph nodes were the same for both groups of mice. Although all subcutaneous tumors eventually demonstrated neovascularization, the rate of development of angiogenesis was slower and the effect clearly less intense in W/W^V mice. The incidence of spontaneous pulmonary metastasis was lower (26.6%) in W/W^V mice compared to the controls (60.0%), as was the number of pulmonary metastases found per mouse. We conclude that host mast cells may have an important role in early tumor angiogenesis and may facilitate hematogenous metastasis. Experiments are in progress to confirm these interpretations using mast cell reconstituted W/W^V mice. NIH-CA 39611, NIH-5S06RR08218-03.

Q 127 METASTATIC VARIANTS OF A HUMAN LUNG CARCINOMA CELL LINE ISOLATED USING ATHYMIC MICE, Nissi M. Varki and Leita Roome, University of California, San Diego, La Jolla, CA 92093.

The metastasis of malignant neoplasms is a significant clinical problem. The death of many patients with cancer is directly or indirectly due to metastases, rather that to the primary neoplasm. The current understanding of the pathogenesis of the metastatic disease process has evolved from extensive studies, most of which utilized transplantable rodent neoplasms. The study of human neoplasms in similar experimental systems was not possible until recently when it was shown that athymic mice may serve as useful models for such studies. This report shows the establishment of such a model system. A human lung carcinoma cell line, UCP3, was carried as subcutaneous xenotransplants in athymic mice. Autopsies of these animals showed rare foci of microscopically visible metastases in the lungs and lymph nodes of 30% of these animals. There were no metastases to any of the other organs. A metastatic variant, 522 was established serendipitously in vitro as a continuous cell line by blind isolation of the pulmonary metastatic foci, at the time of autopsy, from the lungs of the animals that carried subcutaneous xenotransplants of the parental cell line. The parental UCP3 and the metastatic variant 522 were examined by karyotypic and isoenzyme analysis and shown to be human and related. The metastatic variant, 522, metastasizes spontaneously from subcutaneous sites (like the parental UCP3). However, it forms larger subcutaneous xenotransplants than does the parental cell line, and forms many more metastatic foci in the lungs of 80% of the animals. Comparisons of the cell surface glycolipids show many similarities and a few differences. Additional metastatic variants were obtained by passage and selection in athymic mice. This model system may now be used for further investigations on the processes of metastasis of this human neoplasm.

Q 128 IDENTIFYING ALTERED PATTERNS OF DNA METHYLATION IN PRIMARY AND METASTATIC HUMAN LUNG CARCINOMAS, John C. Wain, David J. Baker, John R. Benfield, Steven S. Smith, City of Hope National Medical Center, Duarte, CA 91010

Methylation of cytosine residues in DNA functions as one mechanism for control of gene expression. Alterations in cytosine methylation have been identified in neoplastic cell lines and solid human tumors. We have been studying alterations in methylation state at the genomic level in freshly excised human lung carcinomas. Differential cleavage of genomic DNA by Hpa II or Msp I was followed by Southern transfer of the restriction fragments and hybridization with a ^{32}P labelled probe containing a 3.4 kB sequence from the KPN family. Among the Hpa II (methylation sensitive) restriction digestion fragments of the human lung carcinomas, KPN probe binding was seen at distinct restriction fragment length polymorphisms (RFLPs). Such selective KPN probe hybridization to Hpa II fragments was observed with each of 32 human lung carcinomas studied and with none of 10 samples of non-neoplastic lung tissue studied. Molecular weights were assigned to prominent RFLPs within each Hpa II digestion analyzed. Unique descriminatory RFLP patterns could be identified on this basis for 1. primary non-small cell lung carcinomas, 2. primary neuroendocrine lung carcinomas, and 3. metastatic pulmonary adenocarcinomas. A KPN probe can identify patterns of altered genomic methylation which differ between primary and metastatic human lung carcinomas.

Q 129 ROLE OF ACTIVATED RAS ONCOGENE IN PROGRESSION OF HAMSTER BRAIN TUMORS, Stephen G. Zimmer, Jackie Fetherston, Jenny Cotton, and John Walsh, University of Kentucky, Lexington, Ky. 40503.

Initial studies on the transformation of primary hamster glial cells from different regions of the brain with SV40 virus resulted in the production of a panel of cells with varying degrees of tumorigenic or invasive potential. Cloned cell lines which differed in their ability to invade adjacent normal brain were derived from the transformed cerebral cortex glia cell population. We wished to determine if the introduction of an activated C-Ha-Ras oncogene could progress cells that did not invade normal brain to a full expression of the invasive phenotype.

The T24 bladder oncogene along with pSV2neo was introduced into primary cortex glial cells and into a cloned Sv40 transformed cortex glial cell line which was tumorigenic, but not invasive. Selection of transfected cells with G418 resulted in the production of cell lines derived from both the primary and Sv40 transformed cell populations that contained C-Ha-Ras oncogene as confirmed by Southern blot analysis. Expression of the transfected T24 oncogene was confirmed by Northern and Western blots. These cell lines were then injected intracranially into newborn hamsters. After 3-4 weeks, when the animals exhibited signs of neurological impairment, the animals were sacrificed and the tumors were evaluated for invasiveness into adjacent normal brain. In the case of both the C-Ha-Ras transfected primary and SV40 transformed glial cell lines the lines were found to progress to a full invasive phenotype. Control cells were either non-tumorigenic (primary) or non-invasive (SV40 transformed line) after transfection with pSV2neo and selection with G418.

Differentiation, Invasion and Growth in the Host Environment

Q 200 CONTRIBUTION OF IMMUNOREGULATORY FACTORS TO THE DEVELOPMENT AND PROGRESSION OF PROSTATE CANCER. Richard J. Ablin, Laszlo Muszbek, Janos Polgar, John M. Bartkus and Maurice J. Gonder. SUNY Stony Brook, Stony Brook, NY 11794 and University School of Medicine, 4012 Debrecen, Hungary.

Attention to the participation of immunological aspects of host resistance in the pathogenesis of prostate cancer (PCa) was brought to light by observations of remission of metastases following cryosurgery of the prostate. These observations prompted immunobiological studies of the prostate and of patients (pts) with PCa and provided varying degrees of evidence of host response to tumour. However, the magnitude and consistency of these responses are less than those observed in pts with other solid tumours. Coincident with these observations, there is clinical evidence in PCa pts that there may be factors interfering with tumour-host responsiveness. What then is (are) the perhaps unique characteristics of the prostate responsible for these observations? Escape from immune surveillance in the presence of tumour immunogenicity may lie with factors within the tumour, i.e., its microenvironment (milieu). Investigation of the contributory role of components of the prostate's secretory milieu and possible tumour elaborated factors as a point of departure have permitted identification and presumptive physicochemical characterization of immunoregulatory factors (IRF) in prostatic and seminal fluids and prostatic tissue extracts. These IRF appear to be associated with high and low molecular weight macromolecules. Implicated here are indomethacin and iodoacetamide sensitive molecules, the latter of which may be associated with transglutaminase and the formation of protein-polyamine conjugates.

Q 201 ENHANCED INVASION IN VITRO AND REDUCED LAMININ PRODUCTION IN HIGH METASTATIC LINES OF K-1735 MELANOMA AND IN A V-MYC TRANSFECTED VARIANT.

A. Albini, K. Römisch, G.R. Martin, S.L. Auckerman*, and I.J. Fidler*. LDBA, NIDR, NIH, Bethesda, MD and *Dept. of Cell Biology, M.D. Anderson Hospital, Houston,TX.

Laminin is a basement membrane specific glycoprotein composed of three chains, an A, B1, and B2 chain. Laminin promotes tumor cell adhesion in vitro and the formation of tumor metastases in vivo. Highly metastatic cells of K-1735 mouse melanoma lines show a high degree of invasiveness through reconstituted basement membrane and exhibit higher laminin binding than do low metastatic variants. A non-metastatic, non-invasive clone was cotransfected with a v-myc construct and pSV-neo, a selectable marker. The cells obtained expressed v-myc and were highly invasive. Cells transfected with pSV-neo alone did not acquire the invasive phenotype. We have also investigated these lines for their endogenous production of laminin. The synthesis of laminin was decreased in the highly metastatic cells as well as in the v-myc transformed line as indicated by Western immunoblots of cell lysates and immunofluorescence analysis. The level of laminin B2 mRNA was similar for all the cell lines, whereas B1 mRNA was decreased in the highly metastatic and the v-myc cell lines. None of the lines showed detectable amounts of A-chain protein or mRNA. Since B1 contains the cell binding domain of laminin (Graf et al, in press), we postulate that a decreased production of the B1 chain could result in a greater proportion of free laminin receptors on the cell surface and account in part for the high affinity of the metastatic lines for basement membrane.

Q 202 SPECIFICITY OF ADHESION BETWEEN TUMOR CELLS AND CAPILLARY ENDOTHELIUM: AN IN VITRO CORRELATE OF PREFERENTIAL METASTASIS IN VIVO

R. Auerbach, W.C. Lu, F. Gumkowski, M. Kaminski, L. Kubai, J. Bielich
Laboratory of Developmental Biology, Department of Zoology, University of Wisconsin, Madison, WI 53706

We have studied the tendency of various mouse and rat tumor cell lines to adhere to endothelial cells derived from different organ sources. Some of these tumors -- an ovary-seeking derivative of the OTT 6050 teratoma line, the Morris H 7777 rat hepatoma, and the GL-26 mouse glioma preferentially adhere to ovary, liver and brain derived microvascular endothelial cell monolayers, respectively. Other tumor cell lines (Hoak endothelioma, S180 sarcoma, MBT-2 bladder tumor, BW 5147.3 lymphoma, C755 mammary adenocarcinoma) also show adhesive preferences, but these are not clearly correlated with in vivo behavior of these tumors. Our results suggest that endothelial cell surface-associated specificities may play a significant but not exclusive role in determining the pattern of metastasis.

Q 203 A ROLE OF HOMING RECEPTORS IN MURINE LYMPHOMA METASTASIS
Robert F. Bargatze, Nora W. Wu, Irving L. Weissman and Eugene C. Butcher
Stanford University, Stanford, Ca. 94305

Lymphoid neoplasms display a wide spectrum of growth patterns in vivo, from presentation as a solitary extralymphoid tumor, to disseminated involvement of all lymphoid organs. Since normal lymphocytes are uniquely mobile cells throughout much of their lifecycle, it is reasonable to propose that the dissemination of malignant lymphocytes may reflect their expression of normal lymphocyte "homing" mechanisms. The migration and traffic of normal lymphocytes to lymph nodes is controlled in large part by the regulated expression of surface receptors for high endothelial venules (HEV), specialized venules that mediate the extravasation of circulating lymphocytes from the blood into lymphoid organs and sites of chronic inflammation. To examine whether the expression of functional homing receptors for HEV might also play an important role in the in vivo behavior of neoplastic lymphocytes, we have compared the patterns of growth of several HEV binding and non-binding murine lymphomas following passage into syngeneic recipients. The results demonstrate a clear correlation between the expression of homing receptors and the in vivo metastatic patterns of the transferred lymphomas. HEV binding lymphomas uniformly metastasize by the blood to give gross enlargement of all lymph nodes. Non-binding lymphomas, on the other hand, produce little growth change in distant lymph nodes groups; the predominant manifestation was massive local growth, with enlargement of local lymph nodes draining the site of tumor cell injection.

Q 204 INTERACTIONS OF B16 MELANOMA CELLS WITH MURINE MESENTERY IN VITRO - A MODEL SYSTEM FOR STUDYING TUMOR INVASION.
Paul B. Bell, Tien-Ling Lee, and Ming-Ling Yang.
University of Oklahoma, Norman, OK 73019.

Interactions of B16 melanoma cells with normal target tissues during the early stage of invasion were studied using the murine mesentery in vitro as a model system. Isolated mesentery fragments on rings cut from cytocentrifugation filter cards were cultured in Eagle's Medium supplemented with 0.5% fetal bovine serum and 25mM HEPES buffer in a humid atmosphere of 5% CO_2 and 95% air at 37°C. B16-F10 melanoma cells resuspended in Hank's balanced salt solution (HBSS) were seeded onto mesentery pieces in 24-well plates at a final concentration of $5x10^3$ cells/2ml/well. At various times after seeding, specimens were rinsed with HBSS and fixed for LM, SEM, TEM, and immunofluorescence microscopy. Parallel observations were made by time lapse cinematography. Over a period of 14 hours, there were no detectable changes in the structure of the cells or extracellular matrix (ECM) compared with that of controls in vivo . Within 1/2 hour after seeding, tumor cells were able to adhere to the mesentery at or near the boundaries between mesothelial cells. These tumor cells caused the mesothelial cells to retract, exposing the underlying ECM onto which the tumor cells spread. Some tumor cells penetrated into the mesentery, while others remained on the surface where they grew into nodules. Once inside the mesentery, tumor cells lost most of their microvilli while retaining their ability to move around. No dissolution or distortion of collagen or elastic fibers was observed. Our results demonstrate that the murine mesentery system is a useful and versatile model for in vitro studies of tumor invasion. (Supported by NIH grant 1R01CA36547)

Q 205 EVALUATION OF SPONTANEOUS VARIANTS OF THE JB/MS MELANOMA AS A MODEL OF TUMOR PROGRESSION,
Jane Berkelhammer, Department of Cell Biology, AMC Cancer Research Center, Denver, CO 80214

The present study was designed to evaluate selected phenotypic characteristics of the JB/MS melanoma as a model for the characterization and manipulation of tumor progression. The JB/MS melanoma, which was originally induced in our laboratory with 7,12-dimethyl-benz[a]anthracene (DMBA) and croton oil, has produced a range of spontaneous variant cell populations after propagation in vivo and in vitro. The parent cell line is heavily melanotic, slow-growing and limited in metastasis to the draining and regional lymph nodes. Moreover, histologically, the parent JB/MS tumor resembles a cellular blue nevus, which is generally a nonmalignant lesion in man. The spontaneous variants of the JB/MS melanoma exhibit a range of growth, pigmentation and metastatic properties. Over time, the parent cell line has produced an increasing number of amelanotic tumors. We have found a good correlation between decreased pigment production and increased tumorigenicity, as well as between increased tumorigenicity and increased experimental metastasis in the spontaneous JB/MS melanoma cell variants. A single passage of JB/MS cells through immunized mice has also resulted in cell populations with increased tumorigenicity, growth rate and metastatic abilities. A thorough investigation of phenotypic differences among these spontaneous variant cell populations will provide important information about the biologic and immunologic factors that participate in melanoma growth and progression. Supported by NIH grants CA38110 and RR05894, and a gift to the AMC Cancer Research Center from the Goodstein Foundation.

Q 206 ORGAN SPECIFIC METASTASIS DIRECTED BY THE EXTRACELLULAR MATRIX. R.F. Cerra and R.B. Natale, University of Michigan, Department of Internal Medicine, 3700 Upjohn Center, Ann Arbor, MI 48109-0504.
The biochemical mechanism by which circulating tumor cells reconize and invade organs in a specific manner (organotropism) is poorly understood. We have developed an *in vitro* model system that allows direct biochemical analysis of cell-matrix interactions. When sublines of the B-16 melanoma, which metastasize to specific organs *in vivo* are allowed to adhere to tissue culture plates coated with various organ biomatirces (lung, liver and kidney) derived by high salt extraction and DNAase/RNAase digestion, organ specific attachment is observed. The lung colonizing B16-F10 line preferentially attaches to lung biomatrix while the liver colonizing B16-L46 line specifically adheres to liver biomatrix. Organ specific attachment of the cell lines is retained when guandine extracts of the biomatrices are coated onto tissue culture plates instead of the organ biomatrices. In addition, specific adhesion of the cells to the biomatrix is blocked when cells are pretreated with the guandine extracts. These results suggest an organ specific component which influences tumor cell attachment is present in the guanidine extracts.

Q 207 MODULATION OF B16 MELANAOMA ADHESION, Corrado D'Arrigo and Clive W. Evans, University of St Andrews, St Andrews, Fife, KY16 9TS, Scotland.
B16 melanoma cells adhere and spread on plastic substrates coated with serum proteins (fibronectin, vitronectin) or with proteins from the extracellular matrix (collagen) or basement membrane (laminin). Not all proteins provide an adhesive surface, however, nor do all proteins promote spreading. Although spreading on a 2-dimensional surface cannot proceed without prior adhesion, it is possible to separate the two phenomena operationally in kinetic terms. Thus some proteins induce rapid adhesion (5 min) whereas others do not: fibronectin is the major serum protein which induces rapid adhesion of B16 melanoma cells whereas vitronectin induces spreading but not rapid adhesion. Although serum contains fibronectin it does not induce rapid adhesion. These results suggest that serum components may have different affinities for plastic substrates and/or other components may modulate the adhesive interactions of B16 cells. We have isolated a negatively charged moiety from serum by FPLC that may serve to modulate the adhesive interactions of cells.

Q 208 HETEROGENEITY OF FIBROBLAST RESPONSE IN HOST-TUMOR CELL-CELL INTERACTIONS IN METASTATIC TUMORS, M.Kh. Dabbous, L. Haney, L.M. Carter and A.K. Paul, University of Tennessee, Memphis, Memphis, TN 38163.
The invasive growth of malignant tumors is associated with host tissue degradation which is believed to be due to elevated levels of collagenolytic enzymes. The mechanism of enhanced release of these enzymes, however, is still unclear. Histologic examination of human and animal tumors showed morphological changes in stromal fibroblasts and mast cells at the invasive zone. Numerous mast cells appeared at microfoci along the tumor:host tissue junction and mast cell degranulation was often associated with disruption and lysis of the connective tissue matrix. In vitro studies, using rat mammary adenocarcinoma and human lung adenocarcinoma cells, showed that both tumor and host cells participate in collagen degradation by releasing collagenolytic enzymes. Tumor associated stromal fibroblasts released higher levels of collagenolytic activity than normal fibroblasts and were more responsive to stimulation by tumor-derived cytokines and soluble mast cell products. However, host fibroblasts appear to be a heterogeneous population of responsive and nonresponsive subpopulations based on differences in their response to tumor or mast cell-mediated stimulation of collagenase release. The data suggest that host-tumor cell interactions at the tumor periphery play a significant role in host tissue degradation, however, heterogeneity of stromal fibroblasts may determine the site and extent of tissue damage at foci of tumor invasion. (Supported by NIH Grant CA 25617).

Q 209 LAMININ EXPRESSION IN HUMAN COLORECTAL CARCINOMA CELLS VARYING IN THEIR DEGREE OF DIFFERENTIATION. George Daneker, Lisa Guerra, Glenn D. Steele, Jr. and Arthur M. Mercurio. New England Deaconess Hospital, Harvard Medical School, Boston, MA 02115.
Laminin plays a key role in tumor invasion and progression because these processes require localized basement membrane "disassembly". We are studying the ability of colorectal carcinomas that vary in degree of differentiation to assemble a basement membrane, and to relate differences in this ability to perturbations in laminin synthesis, glycosylation, and secretion. For these studies, we are using human tumor cells grown both in vitro and in nude mice as well as tumors obtained at surgery. Immunoperoxidase staining of paraffin sections obtained from the tumors indicates that laminin is present in a defined basement membrane in moderately and well differentiated tumors. This organized staining pattern is absent in poorly differentiated tumors. In these tumors, the staining is discontinuous and laminin is sometimes observed intracellularly. Similar results were obtained from immunofluorescent staining of tumor cells grown in culture. Moderately and well-differentiated cells assemble an organized matrix that stains for laminin. No such matrix is present in poorly differentiated cells, although significant intracellular laminin staining is evident. The laminin synthesized by the cultured cells was immunoprecipitated and analyzed by SDS-PAGE. Initial results suggest a differential migration of both the cellular and secreted forms of laminin that is related to differentiation. Poorly differentiated cells may contain precursor forms of laminin in larger amounts than well differentiated cells which contain mature forms. We are characterizing these laminin structural differences in more detail. Such differences may contribute to the inability of poorly differentiated tumors to make a basement membrane.

Q 210 INHIBITION OF TUMOR CELLS METALLOPROTEINASES BY A RECOMBINANT HUMAN FIBROBLAST COLLAGENASE INHIBITOR, Yves A. DeClerck, Ofelia Alvarez and David Carmichael, Childrens Hospital of Los Angeles, University of Southern California, Los Angeles, CA 90027 and Synergen Inc., Boulder, CO 80301.
We have investigated the ability of a recombinant collagenase inhibitor (rHCI) obtained from human fibroblasts (Carmichael, et al., P.N.A.S. Vol. 83:2407-2411, 1986) to inhibit the degradation of collagen by human tumor cells in vitro. The recombinant inhibitor has a molecular weight of approximately 20 KD and was found to have inhibitory activity against classical collagenases obtained from human and rabbit fibroblasts and type IV specific collagenase obtained from tumor cells, but had no activity against clostridial collagenase. Gelatin-substrate gel electrophoresis was used to identify several metalloproteinases with gelatinolytic activity in tumor cells extracts and conditioned medium. All but one of these metalloproteinases were inhibited by the rHIC. The inhibitory activity of the recombinant inhibitor on the degradation of radiolabeled collagen matrices by living tumor cells was also investigated. Inhibition of the degradation of interstitial type I and III collagens was observed but only at high concentrations of the inhibitor (10 to 50 ug/ml). Our data indicate that rHIC is a potent inhibitor of tumor cells metalloproteinases and can inhibit tumor cell mediated collagenolytic activity. Whether this inhibitor could play a role in the prevention of tumoral invasion and metastasis has to be determined.

Q 211 CHANGES IN CELLULAR PROTEIN PROFILES DURING AZOXYMETHANE INDUCED COLON CARCINOGENESIS IN F344 RATS FED A LOW OR HIGH FAT DIET, Jose Guillem[1], Michael E. Lambert[2], James I. Garrels[2], Kenneth A. Forde[3], Michael R. Treat[3], and I. Bernard Weinstein[1]
[1]Comprehensive Cancer Center, [1]Columbia University, New York, New York, [2]Cold Spring Harbor Laboratory. Cold Spring Harbor, New York, [3]Dept. of Surgery, Columbia University, New York, New York 10032. We have applied the QUEST system of high resolution two dimensional protein gel electrophoresis, computer assisted quantitative analysis, and protein database development to the study of stage specific changes during azoxymethane (AOM) induced colon carcinogenesis. Animals were initiated with three s.c. injections of AOM (15 mg/kg) over a three week period, and further subdivided one week later into two groups that were fed either a high or low fat diet. Short term explant cultures of normal colon mucosa, adenomas and carcinomas established during subsequent serial sacrifices were then metabolically labeled with S-35- Methionine. Using the QUEST system to quantitate and automatically match gel images, samples from each sacrifice are being assembled into a protein database to compare the levels of synthesis of over 2000 proteins from each radiolabeled tissue sample. Analysis of samples from the first serial sacrifice, collected 5 weeks after the last injection, shows changes in patterns of expression of specific sets of cellular proteins which correlate with either dietary group, carcinogen treatment or both. Both quantitative and qualitative changes, including increases and decreases in rates of protein synthesis, have been scored. We will describe markers of both early pre-neoplastic events and changes occuring at later stages, during the appearance of adenomas and invasive carcinomas.
Supported by NCI Grants CA 02656, CA 13696 and an ACS Institutional Grant to IBW.

Q 212 PREVENTION OF GRAFT-VERSUS-HOST DISEASE (GVHD) FOLLOWING ALLOGENEIC MARROW TRANSPLANTATION (BMT) APPEARS TO ABROGATE THE ANTILEUKEMIC IMMUNOLOGIC SURVEILLANCE OF THE MARROW GRAFT. Henslee PJ, Thompson JS, Romond EH, Doukas M, Messino M, and Macdonald JS.

Twenty historical patients (pts) who received T-replete matched human leukocyte antigen identical (HLA-ID) marrow grafts from siblings (Group 1) are compared with 68 pts who have received marrow grafts from HLA-ID siblings (36 pts: Group 2) or partially mismatched haploidentical (Haplo-ID; 32 pts, Group 3) family members. T-cell depletion of the marrow graft was accomplished by incubation with complement mediated monoclonal antibodies (MoAb) utilizing a single, CD-3 MoAb, T10B9, alone or in combination with a CD-6 MoAb, T12A10; a technique shown to remove 95-99% of phenotypic T-cells assayed with a fluorescent monoclonal antibody conjugate. Patients in all groups received conditioning therapy with combination chemotherapy including high dose cyclophosphamide and cytosine arabinoside, ± methylprednisolone and total body irradiation in doses ranging from 5-14 Gy. All pts were transplanted for an underlying hematologic malignancy. The majority were considered high-risk due to advanced disease status: $^{15}/_{20}$, Group 1; $^{21}/_{36}$, Group II, and $^{29}/_{32}$ Group III. Survival in days post BMT range from 988 to 1289 in Group 1 ($^{6}/_{20}$, 31.5%); 150 to 955 in Group 2 ($^{11}/_{36}$, 30.5%); and 30 to 968 in Group 3 ($^{9}/_{32}$, 28%). In pts receiving HLA-ID BMT (Group I & II) a reduction in GVHD was demonstrated (73.6% vs 44.4%, p=<0.06); however, an expected improvement in overall survival was not gained (31.5 vs 30.5%). A negative impact on survival in Group II patients appears to be related to a significant increase in disease relapse rate (41.6% vs 0%, p=<0.005). These data suggest that abrogation of GVHD through T-cell depletion may preclude a systemic antileukemic, cytolytic surveillance imparted by immunoreactive, mature, donor T-cells and supports the hypothesis that immunogenicity of the marrow graft is important in the control of micro-residual disease post BMT.

Q 213 THE APPEARANCE AND DISAPPEARANCE OF F-ACTIN DURING TUMOR CELL INVASION THROUGH A BASEMENT MEMBRANE IN VITRO, Mary J.C. Hendrix and Elisabeth A. Seftor, Department of Anatomy, University of Arizona, Tucson AZ 85724. The migratory ability of metastatic tumor cells is partially dependent on the composition of the cytoskeleton. Our study examines the role of the cytoskeletal protein - F actin - during tumor cell invasion through a basement membrane (BM) in vitro. The human amnion model was used in combination with fluorescence microscopy. High (A375M) and low (A375P) metastatic variants of a human melanoma cell line were seeded onto dissected human amniotic BMs situated in Membrane Invasion Culture System (MICS) chambers. After 15 hr in culture, aliquots of A375M and A375P cells that successfully invaded the BMs were collected and then plated on plastic for 2 hr. Preinvaders and postinvaders of A375M and A375P cells were fixed in 3.7% formaldehyde/PBS and stained with rhodamine conjugated NBD-phallacidin (Molecular Probes, Inc.) at a final concentration of 1:20/PBS for 20 min at room temperature. Subsequently, the cells were washed in PBS and viewed with a Zeiss fluorescence microscope (std. 18 model). Fluorescence studies reveal somewhat of an organized F-actin cytoskeleton only in the A375P cells before invasion through the amniotic BMs. After invasion the A375P cytoskeleton is extremely disorganized. F-actin is only slightly visible in a disorganized pattern in the A375M cells before invasion, but not visible in the A375M postinvaders. Current studies are underway to determine if new cytoskeletal proteins are being synthesized by the invading tumor cells or if the cytoskeletal proteins are being reorganized during invasion. However, a common feature is that no significant staining for F-actin in either variant line after invasion is observed. Research supported by 1R01CA42475 and the Flinn Foundation.

Q 214 ROLE OF CELL SURFACE GLYCOSYLATION IN METASTASIS OF RAW117 MURINE METASTATIC LYMPHOMA CELLS. S.S. Joshi*, D.D. Welsenburger* and J.G. Sharp+, Departments of Pathology & Microbiology and Anatomy+, Univ. of Nebr. Medical Center, Omaha, NE 68105, K.W. Brunson, Indiana University School of Medicine, Gary, IN 46408.

Using an experimental murine lymphoma metastasis model, consisting of the highly malignant/metastatic RAW117-H10 cell line and its parental less malignant/metastatic RAW117-P cell line, we have studied the role of cell surface glycoconjugates in the metastatic behavior of these cells. Flow cytometric analysis revealed a differential expression of certain glycoproteins and glycolipids on RAW117 lymphoma cells. RAW117-H10 cells, expressed lower levels of non-sialylated glycoconjugates and were resistant to natural killer (NK) cell mediated cytolysis. In contrast, the RAW117-P cells expressed higher levels of non-sialylated glycoproteins and were sensitive to NK cell mediated cytolysis. The metastasis-associated glycoconjugates were non-cytolytically removed from the cell surfaces by short-term treatment with n-butanol. The in vivo malignancy of butanol-treated RAW117-H10 cells was decreased significantly and their NK susceptibility was increased considerably. When butanol-treated cells were cultured in vitro to regenerate their cell surface molecules, they regained their in vivo malignant/ metastatic potential and NK cell resistance. Electrophoretic analysis of the butanol-extracted molecules revealed the presence of four major glycosylated molecules unique to malignant/metastatic RAW117-H10 cells. Thus, our results demonstrate the presence of surface glycoconjugates which appear to strongly influence the metastatic behavior of RAW117 lymphoma cells. (Supported by Nebraska Dept. of Health LB506 funds).

Q 215 REGULATION OF UROKINASE EXPRESSION AND PLASMINOGEN ACTIVATOR INHIBITOR (PAI-1) SECRETION IN HUMAN LUNG CARCINOMA CELLS BY TRANSFORMING GROWTH FACTOR-&.

Jorma Keski-Oja, Edward B. Leof and Harold L. Moses. Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232.

Transforming growth factor & (TGF-&) is a stimulator and an inhibitor of cellular growth that can affect the proteolytic activity of cultured cells by inducing endothelial type plasminogen activator inhibitor (PAI-1) and affecting the secretion of plasminogen activators (PAs). A549 human lung adenocarcinoma cells are very sensitive to TGF&-induced growth inhibition. We studied the relationships between the TGF&-incuded growth inhibition and the effects of TGF& on the secretion of PA activity by A549 cells. PA-activity was quantitated by caseinolysis assays, and characterized by urokinase mRNA analysis, immunoprecipitation and zymography assays. PAI production was observed in autoradiograms of SDS-polyacrylamide gels and reverse zymography assays. It was found that TGF& enhanced the production of PA-acitivity by these cells, in accordance with an enhancement of urokinase mRNA levels. In human embryonic WI-38 lung fibroblasts, on the contrary, TGF& caused a decrease in the expression of urokinase. A concomitant stimulation of PAI-1 production was also observed in A549 cells. Analysis of the extracellular proteins of the cells showed that the majority of PAI-1 was either secreted into the medium or deposited to the growth substratum as inactive complexes. Increased proteolytic activity thus appeared to correlate with TGF& inhibition of soft agar growth in A549 cells. The results suggest that TGF& may play a crucial role in the regulation of the invasive, proteolytically active phenotype of certain lung carcinoma cells.

Q 216 IN VIVO USE OF ANTISUPPRESSOR FACTOR MONOCLONAL ANTIBODY AND IL-2 IN THE TREATMENT OF METASTATIC MURINE FIBROSARCOMA, Benjamin Kim, Patricia Warnaka, Michael Iverson* Dept. of Surgery, Case Western Reserve U. School of Medicine at CMGH Cleveland, OH and *Yale U. School of Medicine, New Haven, CT.

Recently, we developed a rat monoclonal IgM antibody (MAb 14-12) against a soluble murine suppressor factor of the in vitro plaque forming response to sheeps red blood cells (TsF-SRBC). This antibody cross reacts with other soluble suppressor factors without antigen or MHC restriction. We tested this antibody for antitumor activity either alone or in combination with Interleukin-2 (IL-2). METHODS: C57BL6 mice injected on Day 0 with methylcholanthrene induced fibro sarcoma MCA 106 were treated I.P. from Day 3-15 with salt solution (HBSS), MAb 14-12, rIL-2 (Cetus Corp), or MAb 14-12 and rIL-2. On Day 20, all mice were sacrificed and total number of pulmonary metastatic nodules recorded. RESULTS: A significant reduction of pulmonary metastases was noted (60-90%) in the groups treated with MAb 14-12 and IL-2 whereas neither agent alone had significant effects. No enhancement of cytotoxicity or proliferative activity was noted.

	Mean Number of Pulmonary Metastases (n) pvalue:			
Expt. #	HBSS	MB14-12	IL-2	IL-2+MAb14-12
1	144(5)	170(6)N.S.	72(5)N.S.	12(6)p<.05
2	212(7)	226(7)N.S.	211(7)N.S.	86(7)p<.05

CONCLUSION: This is the first demonstration of an in vivo antitumor activity of an anti-TSF antibody used in combination with IL-2, a growth factor capable of expanding all functional classes of T-cells, inlcuding suppressors. Studies are underway to define whether selective abrogation of suppression is the underlying mechanism.

Q 217 EXPRESSION OF THE CELL ADHESION MOLECULES NCAM AND L1 IN TUMOR CELLS WITH DIFFERING METASTATIC POTENTIAL D.Linnemann, A.M.Andersson, A.Raz‡ O.D.Laerum§ and E.Bock The Protein Laboratory, University of Copenhagen, Denmark; *The Weizmann Institute, Israel; §University of Bergen, Norway.

During development cell adhesion molecules participate in migratory and morphogenetic processes. The neural cell adhesion molecule NCAM and the L1 cell adhesion molecule are in brain involved in neuronal and glial adhesion and in neuronal migration.

The metastatic process consists of several steps, e.g. tumor cell detachment from the primary tumor, formation of tumor cell emboli in the blood stream and interactions with host cells or other components at the site of metastatic colonisation. Thus, migratory and adhesive properties of the tumor cells may be of importance for their metastatic capacity. We have, therefore, investigated the expression of the cell adhesion molecules NCAM and L1 in weakly and highly metastasizing variants of two melanoma cell lines, B16 and K1735, and of one glioma cell line, BT4C.

B16 melanoma cells expressed both NCAM and L1 but no difference was observed between the weakly metastasizing B16-F1 cells and the highly metastasizing B16-F10 cells. For K1735 melanoma cells, on the other hand, differences in NCAM and L1 expression were observed. NCAM was expressed by both the weakly metastasizing K1735-C1-16 cells and the highly metastasizing K1735-M1 cells but the polypeptide composition of NCAM varied between the two cell lines. L1 was synthesized by K1735-M1 but not by K1735-C1-16 cells. The non-metastasizing glioma cell line, BT4C, synthesized NCAM whereas the metastasizing variant, BT4Cn, expressed no or only very low amounts of NCAM. L1 was not expressed by the glioma cells.

Q 218 IMPLICATIONS OF DIETARY TYROSINE AND PHENYLALANINE RESTRICTION ON METASTASIS OF MURINE MELANOMA. Gary G. Meadows, Catherine A. Elstad, and Mary E. Jaswin, Pharmacology/Toxicology Program, College of Pharmacy, Washington State University, Pullman, WA 99164-6510. In mice inoculated with B16-BL6 melanoma into the pinna of the ear, tumor progression results in lymph node and lung metastases. Preconditioning mice on a diet restricted in tyrosine and phenylalanine does not inhibit primary tumor establishment or lymph node metastasis but inhibits metastatic tumor growth. This restriction almost totally blocks spontaneous hematogenous spread of the tumor to the lung. Mice that develop lymph node metastasis when fed a normal diet and then are fed a diet restricted in tyrosine and phenylalanine exhibit an intermediate number of lung metastases compared to mice continually fed a normal or the amino acid restricted diet. The lung colonizing potential of individual lymph node tumors from dietary modulated mice was assessed to determine if tyrosine and phenylalanine restriction phenotypically alters the experimental metastatic potential of B16-BL6 melanoma. The stability of the metastatic phenotype after *in vitro* culture in tyrosine and phenylalanine deficient media was also examined.

Q 219 METASTATIC BEHAVIOR OF HUMAN COLORECTAL CANCER CELLS IMPLANTED INTO DIFFERENT ORGANS OF NUDE MICE. Kiyoshi Morikawa, J. Milburn Jessup, Shirley M. Walker, and Isaiah J. Fidler. Depts. of Cell Biology and Surgery, The University of Texas, M.D. Anderson Hospital & Tumor Institute, Houston, TX 77030.

The purpose of these studies was to determine whether the pathogenesis of metastasis by human colorectal carcinoma (HCC) cells is dependent on both the intrinsic properties of the cancer cells and the host microenvironment. Three surgical specimen were obtained from three different patients. Two tumors were located in the colon (Duke's B_2, Duke's D) and the third was from a liver metastasis (Duke's D). The tumors were enzymatically dissociated. Viable single cells were implanted into several organs of different nude mice (i.m., s.c., cecum, spleen). Tumors developed in all sites of implantation. However, distant metastases were observed only in mice with HCC cells growing in the spleen, and the degree of hepatic metastasis correlated with the clinical grade of the implanted tumor cells. Tumors from the musculature, subcutis, cecum, spleen and liver were enzymatically dissociated and cells adapted to growth in culture. The metastatic properties of cells from these various lines was examined. Cells from liver metastases were more metastatic than cells established in culture from tumors growing in other sites.

These results indicate that the metastatic behavior of HCC in nude mice is influenced by both of the nature of the tumor cells and by the organ microenvironment of implantation. Moreover, our present studies suggest that the judicious use of nude mice can allow the isolation of variants cells with different metastatic potentials from heterogeneous HCC.

Q 220 INHIBITION OF B16-F10 MELANOMA EXPERIMENTAL METASTASIS: RECENT APPROACHES, Kenneth Olden, Kazue Matsumoto, Sandra L. White, Kenneth M. Yamada and Martin J. Humphries, Howard University, Washington, DC and NIH, Bethesda, MD.
By employing two different approaches, we have been able to effectively block experimental metastasis of B16-F10 murine melanoma cells following intravenous injection into mice. First, coinjection of cells with Gly-Arg-Gly-Asp-Ser (GRGDS), a synthetic peptide which appears to be a crucial determinant in the interaction of cells with fibronectin, dramatically inhibited (>90%) the formation of lung colonies. Inhibition was not due to cytotoxicity of the peptide and was highly specific since closely related peptides, in which amino acids were either transposed, deleted, or substituted, displayed little or no activity. These results suggest that specific cell surface:fibronectin interactions may be crucial for successful completion of the metastatic cascade. Second, treatment of cells prior to injection with inhibitors of protein glycosylation or oligosaccharide processing (swainsonine, castanospermine, and tunicamycin), also resulted in blockage of lung colonization (>80% inhibition). The effects of glycosylation inhibitors were dose dependent, non-cytotoxic, and were not due to suppression of cellular tumorigenicity. Interestingly, administration of swainsonine to mice in drinking water was also inhibitory for melanoma cell experimental metastasis. Preliminary studies using immune-compromised mice suggest that swainsonine may act by modulation of NK cell activity. Supported in part by NIH and Cancer Research Institute grants.

Q 221 HLA-A,B,C EXPRESSION ON HUMAN UROTHELIAL CELL LINES IS INVERSELY CORRELATED WITH TUMOURIGENICITY IN VIVO. S.S. Ottesen, J. Skouv and J. Kieler. The Fibiger Institute, Copenhagen, Denmark.
Three tumourigenic human urothelial cell lines isolated (Hu961a) or derived by "spontaneous" in vitro transformation (Hu609T, HCV29T) from non-tumourigenic cell lines (Hu961b, Hu609 and HCV29, respectively) failed to react with the appropriate semi-specific HLA-A,B antisera in a complement dependent cytotoxicity test as opposed to the original three non-tumourigenic cell lines. Using the monoclonal antibody W6/32 (MoAb W6/32) directed against the monomorphic part of HLA-A,B,C antigens in a quantitative indirect immunofluorescence test no differences was found between the percentage of stained tumourigenic and non-tumourigenic cells, but the staining intensitet of the latter was significantly higher (5-10 fold). Treatment of the tumourigenic cells with neuraminidase increased the staining intensitet with MoAb W6/32. A case of "spontaneous" in vitro transformation of the non-tumourigenic Hu609 cell line with concomitant induction of tumourigenicity and changes in HLA-A,B,C expression is presented. Finally, suggestive evidence for an inverse relationship between the expression of HLA-A,B,C and the c-myc oncogene will be reported.

Q 222 CHARACTERIZATION OF THE HOST MICROENVIRONMENT FOR IN VITRO LEUKEMOGENESIS, Steve Perkins and Roger A. Fleischman, Univ. of Texas Health Sci. Ctr., Dallas, TX 75235
Long-term bone marrow culture is a useful model for the induction and progression of retrovirus-induced leukemias. The success of this technique depends on the formation of a heterogeneous monolayer of bone marrow-derived adherent cells. In order to identify and characterize the essential non-hematopoietic components of this microenvironment, we have utilized chimeric mice produced by microinjection of bone marrow into W/W mutant mouse fetuses, which are deficient in hematopoietic stem cells. In contrast to traditional chimeras, this technique avoids radiation conditioning and resultant stromal injury, precludes engraftment of non-hematopoietic stromal cell precursors, introduces the donor stem cells very early in normal development, and permits transplantation of allogeneic bone marrow selected for a variety of genetic markers, including the major histocompatibility antigens. Adherent layers from these chimeras have been established and analyzed in situ by double-label immunofluorescence with antibodies directed against a host-specific allotype of the H2-K surface antigen and additional cell lineage-specific cytoplasmic antigens. These studies have identified unique functional cell types in the host microenvironment; for example, the endothelial-like cells (cytoplasmic collagen IV and laminin) and the adipocytes have been definitively identified as host in origin. Therefore, these stromal cells are not derivatives of hematopoietic stem cells or lipid-laden macrophages, respectively, as some have proposed. This experimental approach, which delineates the host environment of long-term cultures, will allow the isolation and manipulation of defined cell types for studies of their role in the regulation of hematopoiesis and leukemogenesis.

Q 223 THE INFLUENCE OF ORGAN ENVIRONMENT ON THE METASTATIC PROPERTIES AND PIGMENTATION OF A MURINE MELANOMA. Janet E. Price, David Tarin*, and Isaiah J. Fidler.
M.D. Anderson Hospital and Tumor Institute, Houston Tx 77030 and University of Oxford, U.K.*
The purpose of these studies was to investigate the influence of the host environment on the metastatic and pigmented phenotypes of the murine K-1735 melanoma. A variant line K-1735 SW-1 isolated from an amelanotic lung metastasis was used. This tumor cell line is highly metastatic and produces lesions in many organs following injection into syngeneic mice. In most organs except the brain the metastases are predominantly amelanotic. Tumor cell lines were derived from different organ metastases in mice injected with K-1735 SW-1 cells, and subsequently reinoculated to examine the metastatic potential and pigmentation patterns. The metastatic phenotype was stably expressed by all metastasis-derived cell lines and was thus heritable, while pigmentation of the metastases was not and appeared to be modulated by the site of tumor growth. Assays for tyrosinase activity showed that K-1735 SW-1 cells in vitro did not produce melanin, nor respond to agents which stimulate melanogenesis in another mouse melanoma, the B16 line. K-1735 SW-1 cells, however, do not constitutively lack tyrosinase, since these cells are capable of producing melanin when growing in certain organ sites. The results show that the normal organ environment may influence the phenotypic expression of melanoma cells, in this instance melanin synthesis. These findings recommend caution in extrapolating the results of in vitro biochemical assays to the properties of tumor cells growing in vivo.

Q 224 BIOLOGICAL CHARACTERIZATION OF MACROPHAGE-RESISTANT 3LL-TUMOR VARIANTS IN VITRO AND IN VIVO. Linda M. Remels and P. De Baetselier, Insituut voor Moleculaire Biologie, Vrije Universiteit Brussel, Sint-Genesius-Rode, België.

Following sequential interactions between syngeneic LK/LPS activated peritoneal macrophages (MØ) and 3LL tumor cells, stable 3LL variants (3LL-R) were isolated which manifest a highly reduced susceptibility to the cytotoxic activity of LK/LPS activated MØ. Unlike the unselected 3LL cells (3LL-S), the 3LL-R cells are resistant to high concentrations of TNF.
We found that TNF is involved in the cytotoxic action of LK/LPS activated MØ since rabbit anti-TNF-antibodies could abolish the killing potential of these effector cells.
Furthermore the resistance of the 3LL-R variants to LK/LPS activated MØ in vitro was reflected by a higher tumorigenic and metastatic potential in vivo. In contrast a highly reduced metastatic spread was observed when injecting the 3LL-R tumor cells in poly I:C treated animals. Moreover poly I:C activated MØ are capable of killing both the 3LL-S and 3LL-R tumor cell lines in vitro. Therefore 3LL-R cells might serve as a tool for the detection of alternative MØ-related cytotoxins which are different from TNF or cooperating with it.
No strict correlation was found between the NK sensitivity of the 3LL-R and 3LL-S cells and their metastatic capacity. Hence activated tumoricidal MØ may play a cardinal role in either the elimination or selection of neoplastic cells.

Q 225 ROLE OF UV-B RADIATION IN THE GROWTH OF MELANOMA. Cynthia A. Romerdahl, Isaiah J. Fidler, & Margaret L. Kripke. Depts. of Immunology & Cell Biology, Univ. of Texas, M.D. Anderson Hospital & Tumor Institute, Houston, TX 77030.

The incidence of cutaneous melanoma in the United States has been steadily rising during the last 50 years. Exposure to the UV-B radiation in sunlight may contribute to the induction and/or progression of melanoma in humans. If UV-B radiation increases the growth rate of melanoma cells in vivo, this would suggest that increased UV-B exposure might contribute to the rise in the melanoma incidence and mortality. The purpose of these studies is to determine whether UV-B irradiation of mice produces local or systemic effects that increase the in vivo growth or metastatic potential of transplanted melanomas. C3H mice were exposed to UV-B (280-340 nm) radiation for various periods of time before or after injection of syngeneic K-1735 melanoma cells into the ear (an irradiated site). The incidence and size of tumors were much greater in mice treated with UV-B prior to the tumor injections. Accelerated tumor growth was also observed in mice given UV-B radiation after tumor implantation compared to unirradiated mice. The UV-B-induced effects on tumor growth may be due to a local effect, such as increased vascularity of the irradiated skin. Alternatively, UV-B irradiation may produce systemic alterations that enhance the growth of the K1735 melanoma. Currently, we are attempting to distinguish between these possibilities by transplanting the melanoma cells into unirradiated animals, irradiated animals, and irradiated animals with shielded ears. Our results to date demonstrate that UV-B radiation can markedly accelerate the growth of melanoma cells in mice. Supported by Grant No. R-812607 from the EPA.

Q 226 CHANGES IN THE BIOCHEMICAL PROFILES OF THE CYTOSKELETAL PROTEINS FROM HUMAN MELANOMA CELLS AS THEY ATTACH TO AND INVADE THROUGH AN AMNION MEMBRANE IN VITRO, Richard E.B. Seftor, Elisabeth A. Seftor, Anne E. Cress and Mary J.C. Hendrix, University of Arizona, Depts. of Anatomy and Radiation Oncology, Tucson, AZ 85724.

An important consideration in understanding the complex and dynamic process of metastasis is the role of the cytoskeletal proteins in the invasive movement of melanoma cells. Tumor cell locomotion into regions of tissues modified by cell secreted enzymes is a major step of invasion, and the ability of tumor cells to migrate (invade) and disseminate through the body (metastasize) is directly related to the composition and architecture of the cytoskeletal proteins. In this regard, we have undertaken a biochemical analysis of possible changes in the cytoskeletal proteins which might occur when cells from high and low metastatic variants of a human melanoma cell line (A375M and A375P, respectively) grown on plastic first attach, then invade through an amnion in vitro. Initial results using Isoelectric Focusing in the first dimension and SDS-PAGE in the second dimension of triton-extracted cells grown on plastic show essentially no difference between A375M and A375P in their biochemical profiles. There is also little difference between A375M and A375P cells when they are removed from the amnion after 24hr and their triton-insoluble extracts are run on SDS-PAGE. We are now correlating these findings with what occurs in the biochemical profiles after invasion through the amnion and plan to look into potential post-translational protein modifications - e.g. protein phosphorylation. Furthermore, we are developing methods to study changes which might occur on the mRNA level.
Supported by NIH 1R01CA42475 to MJCH and NIH Trainingship Grant CA09213 to REBS.

Q 227 THE IMPORTANCE OF CELL-CELL AND CELL-SUBSTRATE INTERACTIONS IN INVASION AND METASTASIS. E. Sidebottom and S.R. Clark, Sir William Dunn School of Pathology, Oxford, OX1 3RE, England

We have isolated a number of variants in mouse melanoma and rat sarcoma systems. The variants have been selected on the basis of either tumorigenicity and metastatic potential in vivo or clonal morphology in vitro.

Correlations have been sought between behaviour in vivo and in vitro characteristics such as adhesiveness to natural and artificial substrates, homotypic and heterotypic aggregation, motility and membrane biochemistry.

We find heterogeneity for clonal morphology within metastatic populations, but have succeeded in isolating relatively stable 'tight' and 'loose' cell lines. The latter are more tumorigenic and metastatic in vivo, more motile in vitro and can be detached from culture surfaces more easily. However no differences in rates of attachment or cell aggregation or disaggregation have been found.

No consistent gross disserences in membrane biochemistry have yet been identified between the tight and loose variants. We have, however, recently detected changes in the pattern of glycoprotein molecules shed into the culture medium by highly metastatic and weakly metastatic cells. The results of recent analyses will be presented at the meeting.

Overall we feel our results suggest that cell substrate interactions are likely to be more important than cell-cell ones.

Q 228 IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF LAMININ IN THE ACINAR BASEMENT MEMBRANES OF HUMAN PROSTATIC CANCER, Akhouri A. Sinha, VA Medical Center, Res. Service and Dept of Genetics & Cell Biology, Univ of Minnesota, Minneapolis, MN, 55417.

Structural integrity of basement membranes (BM) is probably altered in adenocarcinoma of prostate. By localizing laminin, a structural component of the BM, in cancerous and normal prostate tissues, I have evaluated the BM. I used affinity purified rabbit antilaminin antibodies and normal goat anti-rabbit IgG complexed with 10-15 nm gold particles (Janssen, SIPI, Westchester, PA or E-Y Lab., San Mateo, CA). Briefly, the technique involved fixation of cancerous and normal prostate specimens in 3-4% buffered paraformaldehyde, dehydration and infiltration in graded ethanol and LR white or in graded N, N-dimethylformamide and Lowicryl (K4M) (Polysciences, Inc.), infiltrations and embedding in 100% resin at room temperature, 4° and/or -20°C, and polymerization at 4°C with UV light. Following evaluation of thick sections, thin sections were hydrated with phosphate buffer saline (PBS), etched for 2' with 10% H_2O_2, incubated with diluted antibody for 1 to 2 h or overnight at 4°C, rinsed with PBS, incubated 1 h with diluted immunogold, rinsed thoroughly, and examined with a Zeiss 10C electron microscope before and/or after uranyl acetate and lead citrate staining. Both the ultrastructure and laminin localization patterns showed focally lysed BM in some areas of cancerous prostate, but not in other cancerous areas and normal glands. In addition, cancerous prostate stroma contained few macrophages and neutrophils which had phagocytosed, presumably degraded laminin of the BM. The host cells probably transported lysed laminin from prostatic stroma to sera. (Supported by Veterans Administration Medical Research funds.)

Q 229 METASTATIC BEHAVIOR OF HYBRIDOMAS AND MYELOMA IS CORRELATED WITH LECTIN RECEPTOR CAPPING, A.Staroselsky, Y.Leibovici, N.Savion, B.A.Sela, M.Michowitz and N.S.Kosower, Tel-Aviv University, ISRAEL.

The cell membrane is important in the expression of malignancy of tumor cells. We found a correlation between the degree of Con A capping, cell adhesion (to plastic, glass and extracellular matrix) and metastatic capacity in a murine myeloma cell line(NSO) and derived hybridomas (Hybr A, Hybr B). The parent NSO exhibited low capping (5%) and high degree of adhesion (60-75%). Hybr A showed intermediate values (10-15% cells capped and 30-50% cell adhesion). Hybr B showed high capping (40-50%) and low cell adhesion (10%). In addition, agglutination by several lectins was more pronounced in Hybr B than in Hybr A and myeloma. Growth in soft agar was similar for the three lines. Subcutanous inoculation into Balb/c mice resulted in local tumor growth at a similar rate for the three lines. However, incidence of metastatic spread (mainly to spleen,& liver) was much more frequent in the high-capping Hybr B than in Hybr A and NSO (90%, 40%, 15% of mice, respectively), with organs of Hybr B-inoculated mice extensively invaded. Following i.v. cell injection, Hybr B-injected mice developed a rapid, extensive tumor growth in above organs (90% mortality within 40 days). By contrast, NSO-injected mice developed tumors slowly in same organs & in abdominal lymph nodes (50% mortality by 120 days). Thus, certain in vitro cell demonstrable membrane properties are indicative of rapid metastatic spread. High membrane component mobility and poor adhesion may also operate in vivo and facilitate detachment from the primary tumor and/or migration of the malignant cell from the blood vessels to certain organs.

Q 230 ALTERATION IN PATTERN AND PROGRESSION OF METASTATIC DISEASE IN RATS FOLLOWING OCULAR IMPLANTATION OF MAMMARY TUMOR, Szalay,J.,Sabzevari, H., Dept. Biol. Queens College, Kissena Blvd Flushing, NY 11367

Mammary tumor (13762) was implanted on the iris of syngeneic Fischer 344 rats. If tumor filled eyes were left intact, or removed after tumor had spread to the orbit, all rats died 7-9 weeks later. Metastasis was seen in the lungs (12/12), lymph nodes (5/12), body wall, (4/12) heart (3/12), and liver (2/12). Removal of the eye (enucleation) when it had just become filled with tumor prevented the development of overt metastatic disease for 1-2 years (11/11). In parallel experiments, enucleated rats were necropsied 0 days to 26 weeks post-enucleation and small nodules were seen in the lung (10/14) or liver (3/14). Nodules were not seen in rats that had tumor implanted on the posterior surface of the cornea (6/6), had corneal implants and enucleation (3/3), or had been housed in our animal facility for 1-2 years (16/16). When tumor was implanted sc, rats died after 6-7 weeks. Metastasis was seen in lung and lymph nodes (12/12), spleen (1/12) and liver (1/12). Removal of very small flank tumors failed to prevent metastasis and animals died 7-8 weeks later with extensive pulmonary metastasis (10/10). Thus, iridial implantation results in an altered pattern of metastasis. Iridial implantation plus enucleation of tumor filled eyes prevents the development of overt metastatic disease, a phenomenon never obtained following removal of extra-ocular primary tumors.

Q 231 DIFFERENT TYPES OF INVASION OF MALIGNANT HUMAN UROTHELIAL CELL LINES STUDIED IN VITRO. V.Tromholt, J.Kieler and B.Christensen. The Fibiger Institute, Copenhagen, Denmark.

The invasion of human urothelial cell lines has been studied in an in vitro model using aggregates of the cell lines associated with embryonic chick heart fragtment or embryonic and adult mouse heart fragments.

Two types of invasion could be demonstrated, i.e. invasion en bloc and invasion by cellular infiltration.

In the present study the influence of DNA inhibitor (5-Fluorouracil) and microtubuli inhibitor (Nocodazole)on the invasiveness of several malignant cell lines demonstrated individual differences in the type of invasion, indicating that invasion en bloc requires mitotic activity and invasion by cellular infiltration requires locomotion. The tested cell lines could be separated in two main groups, that invaded according to the two types of invasion.

Q 232 MALIGNANCY OF MDCK CELLS BEFORE AND AFTER TRANSFECTION WITH ras ONCOGENE, Frans Van Roy, Gilberte Liebaut, Philip Suffys, Chris Dragonetti, Walter Fiers and Marc Mareel, State University of Ghent, Ghent, Belgium.

MDCK, a well-differentiated epithelial canine kidney cell line, was obtained from 2 different sources. One of these lines produced invasive and metastatic tumors in newborn athymic nude mice. The other was non-tumorigenic in vivo and non-invasive in vitro in confronting embryonic chick heart fragments. The latter cell line was transfected with an activated c-Ha-ras gene, derived from the human bladder carcinoma cell line T24. Formation of large colonies in soft agar was used as a criterion for transformation. The resulting MDCKpT24 cell lines differed from each other by the fraction of cells expressing significant amounts of human p21-ras oncogene product (from 0.1 to 50%). These transformants showed no or only moderate invasion in vitro. In contrast, they all produced tumours in vivo, consisting of a mixture of differentiated cells and dedifferentiated invasive cells. Expression of ras gene in these cell types is under investigation. Metastasis was rare. A control cell line, made resistant to geneticin by DNA transfection, formed only benign cysts in nude mice and was not invasive in vitro. Tumor-derived cell lines were enriched for ras-expressing cells and were very invasive in vitro. Ascites-derived cells included a large fraction of giant multivacuolized cells, expressing high levels of p21-ras protein localized atypically. The malignant properties of the tumor-derived lines will be documented further.

FVR is a Research Associate of the N.F.W.O. PS is a Fellow of the I.W.O.N.L. Research supported by FGWO and ASLK, Belgium.

Q 233 DIFFERENCES IN PRODUCTION OF EGF-LIKE ACTIVITY IN HUMAN COLON CARCINOMA CELL LINES ARE RELATED TO THE DEGREE OF DIFFERENTIATION. L.F. Watkins and A.E. Levine
Dept. of Pharmacology, Baylor College of Medicine, Houston, TX 77030.

Human colon carcinoma cell lines grown in defined media secrete EGF-like activity into the media in a manner related to their degree of differentiation. HCT 116 and C cell lines (Group I) were the least differentiated and produced the highest levels of EGF-like activity (150ng EGF/mg protein). The MOSER cell line (intermediate in differentiation) produced less EGF-like activity, 49ng EGF/mg protein. The CBS (Group III) cell line was the most differentiated and produced the least, 10ng EGF/mg protein. However, if HCT 116 or MOSER cells were treated with differentiation agents, such as retinoic acid (RA) or DMF, the amount of EGF-like activity increased (i.e. MOSER 29ng/mg protein; with 0.5% DMF 75ng/mg protein; with 1uM RA 120ng/mg protein). Furthermore, in HCT 116 cells the levels of EGF-like activity varied with growth rate. HCT 116 cells grown in media with transferrin, insulin, T_3, hydrocortisone and Na selenite have a slightly faster growth rate than HCT 116 grown only in basal media lacking these components with a corresponding difference in EGF-like activity, 147 and 20ng/mg protein respectively. This increased production of EGF-like activity was due to the effect of insulin or transferrin which appeared to be additive at low concentrations of insulin (1ug/ml) or transferrin (0.1ug/ml). HCT 116 cells grown initially with insulin and transferrin showed a reduced production of EGF-like activity with the removal of these compounds. Therefore, the differentiation state of the cell as well as the growth conditions appear to be important in the regulation of EGF-like activity produced by human colon carcinoma cell lines. (Supported by NCI grant CA38100 and ACS grant BC-492).

Q 234 LYMPHOCYTE HOMING RECEPTORS: A MODEL FOR METASTASIS. Nora W.Wu, Michael Link, Catherine Carswell, Steve Smith and Eugene C. Butcher, Stanford University, Stanford, Ca. 94305.

Hermes-1 is a rat monoclonal antibody that recognizes a 90Kd glycoprotein on the surface of normal circulating lymphocytes. This putative "homing" receptor adhere to specialized high endothelial venules (HEV) and thus directs the egress of lymphocytes from the circulation into lymphoid organs and other structures. Preliminary studies with Hermes-1 on patient bone marrows at diagnosis (N=32) show a remarkable direct correlation between Hermes-1 expression and degree of clinical lymphadenopathy.All patients with large amounts of lymph node involvement express Hermes-1 antigen on their malignant cells and conversely, patients with negative or very low amounts of Hermes-1 staining have no adenopathy. Hermes-1 also appears to be involved in the skin metastasis of Mycosis Fungoides (MF) and skin lymphomas (12/12 MF and 8/8 skin Lymphomas are Hermes-1 positive). Hermes-1 expression therefore appears to be an excellent marker of lymphoid metastasis and may be useful therapeutically and for prognosis.

Q 235 DIFFERENTIAL PRODUCTION OF HIGH MOLECULAR WEIGHT SULFATED GLYCOPROTEINS IN NORMAL COLONIC MUCOSA, PRIMARY COLON CARCINOMA, AND METASTASIS, Takao Yamori, Hitomi Kimura, Kendal Stewart, David M. Ota, Karen R. Cleary, and Tatsuro Irimura, Department of Tumor Biology, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston TX 77030

Sulfated macromolecules synthesized in tumor and mucosa tissues derived from cololectal cancer patients were labeled with [^{35}S]sulfate and separated into two fractions on DEAE- Sephacel: the slightly acidic peak (peak I) was eluted with 0.2 M NaCl and the highly acidic peak (peak II) was eluted with 0.5 M NaCl. A total of 40 specimens, which included primary colon cancer, liver metastases, and normal mucosa obtained at surgery (16 specimens), were examined regarding the amount of peak I and peak II. the amount of peak I significantly decreased in the order metastases > primary tumors > normal mucosa, while the amount of peak II did not significantly change among the tissues. Peak I was mostly resistant to chondroitinase ABC and nitrous acid treatment under acidic conditions, whereas combined chondroitinase-sensitive materials and nitrous acid-sensitive materials were greater than 80% of the radioactivity in peak II. The major radioactive compornent of peak I migrated at a position corresponding to molecular weight (M_r) > 300k by SDS-polyacrylamide gel electrophoresis and became M_r less than 40k after alkaline borohydride treatment. The major component of peak I was likely to be a sulfated glycoprotein containing sulfated groups on alkaline labile carbohydrate chains. Peak II consisted of a mixture of heparan sulfate proteoglycans and chondroitin sulfate proteoglycans. Decreased peak I production may be a biochemical marker associated with colorectal cancer progression and metastasis. (Supported by USPHS grant RO1-CA39319)

Detection and Therapy

Q 300 A NOVEL APPROACH TO THE EVALUATION OF TUMOR BURDEN IN SOLID ORGANS OF EXPERIMENTAL ANIMALS. Y. Barnavon, H. Iwaki, M. Wallack and J. Bash, Mount Sinai Medical Center, Miami Beach, Florida 33140.
We have employed the intrasplenic injection method of LaFreniere and Rosenberg (JNCI, 76:309, 1986) to produce hepatic metastases of cultured colon adenocarcinomas (CC26, CC36) in syngeneic Balb/c mice. Since these tumors grew in the liver as large confluent masses rather than small discrete surface nodules they were not amenable to surface counting as used by previous investigators to evaluate tumor burden. A novel approach to evaluation of tumor burden was therefore developed. Cultured tumor cells are directly injected into the spleen and embolize via the splenic vein to the liver. A splenectomy is subsequently performed. Following immunotherapy and just prior to sacrifice, an India ink suspension is injected i.v. to stain the normal liver parenchyma and, once harvested, the livers are immersed in Fekete's solution for 30 minutes to bleach tumor masses and fix tissue. Each liver is weighed, placed on an inverted Petri dish lid and compressed between the lid and Petri dish bottom into a uniform thickness of approximately 1mm. The tumor and normal parenchymal surface areas are then delineated with a marking pen on the exterior surfaces of the Petri dish, and a Carl Zeiss Videoplan area reader is used to compute the aggregate surface areas of each. Since the liver is compressed into a uniform thickness, the ratio of tumor area to total area approximates the ratio of tumor volume to total organ volume. Multiplying liver weight by this ratio yields a calculated tumor weight. This method is currently being used to quantify efficacy of LAK/IL-2 immunotherapy but may be applied to other animal models where evaluation of tumor burden in solid organs is derived.

Q 301 EVIDENCE FOR IMMUNOLOGICAL MECHANISMS INVOLVED IN STABILIZATION OF MELANOMA METASTASES IN A PATIENT UNDERGOING ACTIVE IMMUNOTHERAPY WITH VACCINIA MELANOMA ONCOLYSATE (VMO). J.A. Bash, Y. Barnavon, M.K. Wallack, Mount Sinai Medical Center, Miami Beach, Florida 33140.
Patient G.L. presented with a scalp lesion which was biopsied and confirmed as malignant melanoma. After surgery he received weekly injections of VMO at 4 subcutaneous sites for one year and then every other week for a second year. During the course of treatment two nodal lesions were biopsied and diagnosed as melanoma metastases. At the same time splenic metastases were observed by CT scan. The appearance of antibody reactive with melanoma cell lines was detected after 3 months of treatment using a Staphylococcus protein A (SpA) rosette assay. The second nodal lesion regressed rapidly when this circulating antibody titer was high. The splenic metastases remained stable. At this time cellular reactivity to melanoma antigens was also demonstrated by proliferation (3H-thymidine incorporation) of peripheral blood mononuclear cells (PBM) after 5 days of co-culture with melanoma cell lines. Melanoma restricted cytolysis (51-Cr release) was demonstrable after expansion of primed PBM with interleukin-2 (IL-2). Flow cytometry analysis of lymphocyte subsets showed high levels of activated T cells and NK cells. Further studies of antibody production by G.L. confirmed that both IgG and IgM antibodies were restricted to melanoma-associated antigens. Most significantly, immunoperoxidase staining of the patient's own tissue with the patient's antibody showed melanoma-restricted reactivity. Since this same serum had high activity against melanoma cells in a lymphocyte-dependent antibody assay (LDA) in vitro, it is proposed that both cellular and humoral mechanisms may have contributed to stabilization of this patient's disease for over three years after diagnosis.

Q 302 PULMONARY CTL AND MACROPHAGE ACTIVITY CORRELATE WITH THERAPEUTIC EFFECT OF RM IFN G FOR LUNG METASTASES, Paul L. Black, Hamblin Phillips, Henry Tribble, Robin Pennington, Mark Schneider and James Talmadge, Preclinical Screening Lab., Program Resources, Inc., NCI-Frederick Cancer Research Facility, Frederick, MD 21701.
The mechanism of therapeutic activity of rm IFN g in treatment of metastatic disease was investigated by comparing anti-tumor immune responses (both specific and nonspecific) at several sites in mice bearing experimental lung metastases (B16-BL6 melanoma). After both 1 and 3 weeks of rm IFN g administration (iv, tiw), effector functions in spleen, peripheral blood and the tumor-bearing organ (lung) were tested: NK, LAK, CTL against specific and nonspecific targets, and macrophage tumoricidal and tumoristatic activity. Compared to saline, rm IFN g increased survival and decreased the number of metastatic foci in the lungs, at an optimal dose of 50,000 U/animal. Specific CTL activity (against B16, but not against control targets) in lungs, but not in spleen or blood, correlated with therapeutic activity (increased survival and decreased number of lung metastases). Macrophage tumoricidal activity from collagenase-dissociated lungs, but not from alveoli, correlated with therapeutic activity (decreased number of lung metastases). However, NK and LAK activity in any site did not correlate significantly with therapeutic activity. These results suggest that, while immunological monitoring may help predict therapeutic responses and optimize treatment protocols, current monitoring techniques in readily accessible sites may not accurately forecast therapeutic efficacy of BRM's during clinical trials. Research supported by DHHS contract No. N01-23910 with Program Resources, Inc.

Q 303 REVERSAL OF THE TRANSFORMED PHENOTYPE OF MOUSE EMBRYONIC AKR-MCA CELLS MAY DEPEND ON A REDUCTION OF CELLULAR SPERMIDINE. Douglas Boyd, Stan Bialoski and Michael G. Brattain, Bristol-Baylor Laboratory, Dept. of Pharmacology, Baylor College of Medicine, Houston, TX 77030.

The effect of 2 polyamine inhibitors, difluoromethylornithine (DFMO) and dicyclohexylammonium sulfate (DCHS) on cellular morphology, growth in soft agarose and intracellular polyamines was investigated in the transformed fibroblasts AKR-MCA and their parental counterparts AKR-2B. In monolayer both agents promoted a more benign phenotype in AKR-MCA cells closely resembling that of untreated AKR-2B fibroblasts. AKR-MCA cells could be rescued from the effects of these agents by concomittant incubation with exogenous putrescine and spermidine. In soft agarose, transformed fibroblasts exposed to DFMO and DCHS did not form colonies, this effect was reversed by co-cultivation with putrescine. Analysis of cellular polyamines revealed inhibitory effects on spermidine levels; both DFMO and DCHS reduced spermidine to 20-30% of control values by 24h; this molecular effect preceded the accrual of morphological changes in AKR-MCA cells. Dimethylformamide, another agent known to "normalize" the malignant phenotype of AKR-MCA cells also induced similar reductions in intracellular spermidine. Inasmuch as these 3 agents are cytostatic on AKR-MCA and AKR-2B cells, the differentiative effect of DFMO, DCHS and DMF may be secondary to growth inhibition in AKR-MCA cells brought about by spermidine depletion.

Q 304 RECOMBINANT HUMAN GM-CSF ACCELERATES NEUTROPHIL AND PLATELET RECOVERY FOLLOWING AUTOLOGOUS BONE MARROW TRANSPLANTATION. R.E. Donahue, S. Karlsson, S. Clark, W.F. Anderson and A.W. Nienhuis. Genetics Institute, Cambridge, MA, and the National Heart, Lung, and Blood Institute, Bethesda, MD.

Complications associated with bone marrow transplantation often occur during the period of pancytopenia while the bone marrow is regenerating. We have explored the use of recombinant human GM-CSF (rhGM-CSF) in accelerating hematopoietic recovery, using a primate model for autologous bone marrow transplantation. Rhesus monkeys received 1200 rads of total body irradiation and then were infused with 1-4 x 10^8/kg previously collected autologous bone marrow mononuclear cells. Five animals were treated with rhGM-CSF beginning 36-60 hours after marrow infusion; three had also received the hematopoietin for 10-19 days before transplantation. rhGM-CSF was given by continuous intravenous infusion at a dose of 50 units/kg/min. The neutrophil count reached 1000/mm^3 by 8-9 days post-irradiation compared to day 17 and day 24 for concurrent controls. Pre-treatment appeared to have no effect on the rate of recovery. When the rhGM-CSF was discontinued, there was a prompt fall to values comparable to that observed in the untreated control followed by regeneration to normal values. The platelet count of the treated animals exceeded 100,000/mm^3 by days 14-18 compared to day 21 or day 26 for the control animals. Two additional animals were treated with rhGM-CSF 30 or 45 days post-transplantation because of persistent pancytopenia. Each of these animals exhibited a prompt increase in neutrophil and platelet counts to normal values. Our results suggest that rhGM-CSF will prove clinically useful in accelerating bone marrow regeneration.

Q 305 HUMAN TUMOR METASTASIS MODELS IN NUDE MICE, Øystein Fodstad, Institute for Cancer Research, 0310 Oslo 3, Norway.

Experimental metastasis models involving altogether 6 human melanomas, sarcomas and lung carcinomas have been developed in nude mice. Following iv injection of single cell suspensions obtained from xenografts, a tumor specific pattern of metastasis is seen in the animals with metastasis formed in the lungs, lymph nodes, heart, in brown fat and adjacent to the adrenals. The tissue specificity of each tumor line seems to reflect that of the respective tumor type known from the clinic. Upon injection of 10^6 cells from one melanoma line lung colonies are formed that kill the mice in 25-30 days. In contrast, cells from another line selectively give rise to lymph node metastasis, although with both lines the same number of cells were retained in the lungs of the animals during the first 24 hrs after injection. Attempts to select sublines with increased abilities to form metastasis in specific tissues have yielded unexpected results. In vitro cultivated cells have in some cases a reduced metastatic potential, compared to cells obtained directly from sc xenografts. Factors influencing the metastatic ability and tissue specificity of the tumors will be discussed.

Q 306 LOCAL ADMINISTRATION OF INTERLEUKIN-2 ACTIVATES LYMPHOCYTES FROM TUMOR BEARING MICE TO RECRUIT HOST IMMUNOREACTIVITY AND INHIBIT TUMOR GROWTH, Guido Forni, Mirella Giovarelli and Angela Santoni, Inst. of Microbiology, University of Torino, 10126 Torino Italy. Daily administration at the tumor challenge site of 10 injections of recombinant interleukin-2 (rIL-2) induces a consistent, though limited inhibition of the growth of CE-2, a poorly immunogenic chemically induced tumor. By contrast, almost complete inhibition is observed when these injections are performed in mice challenged with CE-2 tumor cells admixed at 1:5 cell ratio with T-enriched lymphocytes obtained from tumor bearing animals. The host immune system plays a fundamental role in this lymphokine activated tumor inhibition (LATI), which is derived from the local combination of rIL-2 and non-reactive lymphocytes. When the host is sublethally irradiated, or the reactivity of $L3T4^+$ and Asialo $GM1^+$ lymphocytes is suppres sed by in vivo antibody treatment, in fact, LATI no longer takes place. Pretreatment of recipient mice with Cyclosporine A or with anti Interferon-gamma monoclonal antibody has the same effect, indicating that lymphokine release and Interferon-gamma in particular plays an important role in the recruitment of host reactivity. The morphological data show that when LATI is taking place the tumor challenge area becomes infiltrated by mononuclear cells and eosinophils, which establish close contacts with each other and with tumor cells. Tumor draining lymphnodes display extension of cortical and paracortical areas. Lastly, the growth of a second contralateral CE-2 challenge is impaired during and after LATI.

Q 307 ISOLATION OF RARE METASTATIC HUMAN NEUROBLASTOMA (NB) CELLS FROM BONE MARROW (BM) BY TWO-COLOR IMMUNOFLUORESCENCE AND CELL SORTING. Christopher N. Frantz and Daniel H. Ryan, Departments of Pediatrics and Pathology and the Cancer Center, University of Rochester Medical Center, Rochester, NY 14642

Two-color immunofluorescence was used to identify and enumerate human NB cells among human BM cells. Monoclonal antibodies that bind to human NB cells, but not to human BM cells were conjugated to fluorescein. Monoclonal antibodies that bind to BM cells but not to NB cells were conjugated to phycoerythrin. When stained with a mixture of these conjugated antibodies, NB cells had green and BM cells red fluorescence. LA-N-1 cultured human NB cells were serially diluted into human BM mononuclear cells. The cell mixtures were incubated with a mixture of phycoerythrin-conjugated anti-BM monoclonal antibodies and fluorescein-conjugated anti-NB antibodies. Using the Epics C flow cytometer with excitation at 488 nm, green and red fluorescence intensity were collected. Forward angle light scatter gates were set to exclude debris and large cell aggregates. In cell mixtures, neuroblastoma cells were reliably identified when they constituted 0.01% or more of bone marrow cells. Background (false positive normal BM cells) range from 0 to 30 cells per million (n=9). When applied to bone marrow containing morphologically identifiable metastatic NB, metastatic cells were identified in 7 of 7 cases. Otherwise undetectable NB was identified in BM from some patients. NB cells were isolated from BM by two-step cell sorting. Use of a second fluorescent color to identify normal BM cells markedly increased the sensitivity of detection. This technique may be used to identify rare micrometastatic solid tumor cells in bone marrow and isolate them for further study.

Q 308 TUMOR PROGRESSION IN THE HUMAN MELANOCYTIC SYSTEM, Meenhard Herlyn, The Wistar Institute, Philadelphia, PA 19104. We have characterized in vitro human melanocytes from normal skin, non-malignat pigmented lesions, and primary and metastatic melanoma. The growth properties of cells in culture and in experimental animals were characteristic for each stage of tumor progression, with metastatic melanoma cells growing at highest densities and plating efficiencies in soft agar. Marked differences in growth requirements for exogenous growth factors were observed even in cell lines derived from primary and metastatic tumors of the same patients which had the same non-random chromosomal abnormalities of chromosomes 1, 6, and 7 and a similar surface antigenic phenotype. Whereas metastatic melanoma cells could be adapted to grow in medium depleted of any proteins of polypeptides, primary melanoma cells required at least one, and mostly several, mitogenic growth factors such as insulin/somatomedin C or EGF. Primary melanoma cells also had more complex substrate requirements. Quantitative differences were found between cells of different stages of tumor progression in the expression of receptors for EGF, NGF, PDGF, and FGF. Our studies indicate the presence of an autocrine growth stimulation mechanism in metastatic melanoma cells that is absent in primary cells.

Q 309 EFFECTS OF TRANSFORMING GROWTH FACTORβ, N,N-DIMETHYLFORMAMIDE AND RETINOIC ACID. N.M. Hoosein, M.K. Matthews, and M.G. Brattain, Bristol-Baylor Laboratory, Dept. of Pharmacology, Baylor College of Medicine, Houston, TX 77030

Effects of transforming growth factorβ (TGFβ) on cellular differentiation have been reported to be stimulatory in some cell types, but, inhibitory in others. In this study, we have examined the effects of TGFβ on two moderately-differentiated colon carcinoma cell lines (JVC and MOSER) and compared them with those of well-known differentiation inducers. N,N-dimethylformamide (DMF) and retinoic acid (RA). JVC and MOSER cells appeared enlarged, more flattened and adherent to the substratum after treatment with 5ng/ml TGFβ. These morphological changes were similar to those induced by DMF and RA. The anchorage-dependent growth of both cell lines was inhibited half-maximally at 1ng/ml (40pM) TGFβ. In JVC cells, inhibition in cellular proliferation by TGFβ, DMF and RA was accompanied by increased fibronectin expression and both these effects were time and concentration dependent as well as reversible. Five fold increased cell-associated fibronectin was found in JVC treated with 5ng/ml TGFβ for 3 days. MOSER cells adapted to grow in serum-free medium display increased levels of media fibronectin in response to TGFβ and DMF but not to RA up to concentrations as high as 10 uM. The data suggest that whilst the two colon carcinoma cell lines are sensitive to the growth-reducing and differentiation promoting effects of TGFβ they show differential sensitivites to DMF and RA. Supported by NIH grants CA38173 and CA34432

Q 310 SYNTHETIC PEPTIDES TO THE B1 CHAIN OF LAMININ WITH ANTIMETASTATIC ACTIVITY, Y. Iwamoto, J. Graf, H.K. Kleinman, M. Sasaki, Y. Yamada, G.R. Martin, NIH, NIDR, LDBA, and F. Robey, Center for Drug and Biologics, FDA, Bethesda MD 20892.

Laminin increases the invasive and metastatic activity of malignant tumor cells. These effects are presumed to be mediated via specific receptors on the surface of the tumor cells. We have cloned the chains of laminin and deduced the amino acid sequence of the B1 chain. Using synthetic peptides and peptide -specific antibodies to the B1 chain, we identified the specific sequence, YIGSR, as the major attachment site in laminin (Graf et al, in press). Here we have tested this and other peptides corresponding to various sequences in the laminin B1 chain for their ability to alter the invasive and metastatic activities of tumor cells. Our in vitro invasion assay tests the ability of tumor cells to migrate across a basement membrane matrix coated onto a filter. Synthetic peptides containing YIGSR, as well as YIGSR alone, prevented melanoma cells from crossing the basement membrane barrier while other peptides from the laminin sequence were not active. Parallel studies examined the effect of these peptides on experimental metastases by tumor cells injected into syngeneic animals. The incidence of lung metastases was reduced by greater than 90% two weeks after co-injection with peptides containing YIGSR. Peptides from five to nine amino acids were active at 100 μg/animal while other peptides were much less active including RGDS, the attachment sequence of fibronectin. These studies suggest that the YIGSR sequence competes with laminin for cellular receptors and that the interaction of the tumor cells with laminin via this receptor is a critical step in the metastasis of the tumor cells.

Q 311 EVIDENCE THAT DELAYED HYPERSENSITIVITY IS REQUIRED FOR REJECTION OF INTRAOCULAR TUMORS. B. Ksander and J.W.Streilein, U.Miami Sch of Med., Miami, FL.

Allogeneic tumor cells(P815 mastocytomas), injected into the anterior chamber(AC) of mouse eyes, enjoy immune privilege: tumors form at this site, whereas no detectable tumors emerge at subcutaneous injection sites. The privilege proves to be relative and dependent upon the degree of immunogenetic disparity between tumor cells and host, i.e. minor H-alone incompatible tumors grow progressively (BALB/c mice), but MHC-incompatible tumors grow transiently and are then rejected (A/J and C57BL/6 mice.) To examine the cellular basis for this difference in growth patterns, delayed hypersensitivity(DH) was measured in mice bearing intraocular tumors; in addition, cells were recovered from tumor-containing eyes at various times post inoculation (p.i.) and then analyzed by limiting dilution for the presence of tumor cells and cytotoxic T cell precursors (pTc). P815-bearing BALB/c eyes yielded progressively increasing numbers of viable tumor cells, despite the detection of pTc within these eyes at seven days p.i. These mice displayed reduced DH responses which are characteristic of the immune privileged state. By contrast, increasing numbers of tumor cells were harvested from tumor containing eyes of MHC-incompatible mice only during the first 14 days p.i. pTc appeared in these eyes at day 7, were present during tumor rejection (days 14-16) and persisted long after. While the initial DH responses of MHC-incompatible recipients were reduced, a strong DH response then emerged, coincident with the intraocular rejection process. We conclude that alloantigen-specific pTc infiltrate intraocular allogeneic tumors, irrespective of immunogenetic disparity. However, successful rejection of the tumor awaits the induction of DH which is essential for in situ pTc activation.

Q 312 URINARY ASSAY OF RAS ONCOGENE PRODUCT IN HUMAN BLADDER CANCER.
Brian C.S. Liu, Michael T. Macfarlane, Jean B. deKernion, and John L. Fahey.
UCLA School of Medicine, Los Angeles, CA 90024.

Although insertion of the ras oncogene may lead to neoplastic transformation or its maintenance, the relationship of such alterations to tumor phenotypic progression is still unclear.

Recently, a sandwich or antigen capture ELISA was developed to detect the presence of ras gene product in urine samples. In this assay, the first layer on the solid matrix was murine monoclonal antibodies against a conserved region of the ras protein (amino acids position 29-44). The second layer was test samples containing the ras antigen. The third layer was sheep antibodies with specificity against the ras antigen. The presence of the third layer was monitored by enzyme conjugated antibodies directed against the sheep immunoglobulin.

Using this sandwich ELISA in a study with 120 concentrated urine samples, 66 controls and 54 patients with urologic cancer (26 active and 28 in remission), 69% of patients with known active malignancy as compared to 9% of controls or 39% of patients in remission have elevated p21 ras expression ($p < 0.001$). Immunoblotting analysis of positive urine samples revealed a band with molecular weight of 21,000 daltons. The increased proportion of high expression among patients thought to be in remission as compared to controls might represent patients with preclinical evidence of a more advanced stage or recurrence.

Q 313 Therapy of Metastasis for Canine Osteosarcoma. E.G. MacEwen, B. Smith, I. Kurzman, P. Manley, R. Rosenthal, School of Veterinary Medicine, University of Wisconsin, Madison, WI

Canine osteosarcoma is spontaneous malignancy in dogs which is characterized by micrometastases to pulmonary and extrapulmonary tissues at the time of diagnosis. The standard treatment involves amputation of the affected leg but the median survival time is 90 days with death due to pulmonary and extrapulmonary metastases. We have been conducting a randomized, double-blind trial, to evaluate liposome encapsulated muramytripeptide (MTP) as a treatment for metastases in dogs undergoing amputation for osteosarcoma.

To date we have entered 18 dogs in the study. Dogs were treated with MTP at a dose of 2 mg/m^2 I.V. starting 24 post amputation and treatments were given twice weekly for 8 weeks (total of 16 injections). The liposome (phosphatidylserine and phosphatidylcholine; PS:PC:3:7 mole ratio) were given at a dose of 20 μ mole phospholipid/Kg body weight twice weekly. Nine dogs have been treated by empty liposomes (placebo) and nine dogs have been treated by liposomes with MTP. The median survival time for the dogs in the placebo group is 82 days with two dogs alive at 70^+ and 251^+ days. The median survival time for the MTP group is 175 days with 5 dogs still alive at 70^+, 76^+, 149^+, 227^+ and 261^+ days. Although these results are preliminary they indicate the liposome encapsulate MTP may have potential for the treatment metastases.

Q 314 HETEROGENEITY OF ONCOMODULIN EXPRESSION IN SOLID TUMORS PRODUCED BY RETROVIRALLY TRANSFORMED CELLS. J.P. MacManus, L.M. Brewer, and M.F. Gillen. National Research Council, Ottawa, Canada K1A OR6

The 11.7kDa calcium-binding protein, oncomodulin, is present in the trophoblast of the placenta of human, rodent, and other mammals. Trophoblast has several properties akin to neoplastic cells: invasiveness, extravasation, and deportation to distant organs. Following neoplastic transformation, oncomodulin has been found in a wide variety of human tumors in >75% of cases (bladder, brain, breast, cervix, colon, kidney, liver, lung, skin and uterus). Oncomodulin can be detected in virally (B77-ASV, KiMSV, SSV, and osteosarcoma virsu) transformed NRK cells. On the other hand, other strains of virally eg. PRC-ASV, SRD-ASV, transformed NRK cells have no detectable oncomodulin. However when solid tumours formed in athymic mice from these negative cells were screened, oncomodulin mRNA and protein were detected. In addition, oncomodulin staining was found immunohistochemically in some tumour cells, in a consistently variable patchy or scattered pattern. Thus the formation of the tumours in vivo has somehow caused either the selection of some cells that express oncomodulin, or the stimulation of de novo synthesis in some cells. To know whether these oncomodulin positive cells in the primary tumour acquire some trophoblastic/tumour-like property of invasiveness or metastasis would be of interest.

Q 315 Relationship between MBr1 monoclonal antibody reactivity and tumor progression. S. Ménard, R. Agresti, M.G. Da Dalt, F. Rilke*, M.I. Colnaghi. Experimental Oncology E, *Anatomical Pathology, Istituto Nazionale Tumori, Milan, Italy.
The MBr1 monoclonal antibody recognizes an epitope (CaMBr1) whose expression seems to be associated with the functional status of the human breast gland. The distribution of CaMBr1 on ducts and lobules of mammary glands tested during the menstrual cycle, pregnancy and lactating demonstrated a fluctuation of antigen expression. MBr1 reactivity in breast carcinomas was found to correlate with the premenopausal status of the patient and the presence of estrogen and transferrin receptors in the tumor. In a retrospective study, carried out on paraffin sections of primary tumors from patients with more than 5 years of follow-up, the "disease free survival" and the "overall survival" were found to be significantly lower ($p < 0.01$) in the group of patients with MBr1 positive tumors. The expression of CaMBr1 in a tumor therefore seems to be associated with a poor prognosis. The in vitro study of MBr1 expression on the breast carcinoma cell line MCF7 shows a heterogeneous pattern of reactivity. By facs-sorting experiments, strongly MBr1 positive cells were found to belong to the proliferative subpopulation and moreover MBr1 antibody was demonstrated to stimulate thymidine incorporation even though in only a very narrow range of concentration . The association between CaMBr1 and transferrin receptors on these cells is now being investigated. Partially supported by grant no. 85.02067.44.

Q 316 SPONTANEOUS REGIONAL LYMPH NODE AND PULMONARY METASTASES AFTER RADIATION OF PRIMARY MELANOMAS, S. David Nathanson, Min Lee, Sherry Vymazal and Fred W. Hetzel. Departments of Surgery, Pathology and Neurology, Henry Ford Hospital, Detroit, MI 48202.

Do subcurative doses of radiation affect the metastatic potential of melanomas? This question was addressed in a spontaneously metastasizing model of the F10 variant of the B16 melanoma in C57BL/6 mice. $5x10^4$ viable F10 cells were injected subcutaneously into the left foot pads of 6-8 week old female C57BL/6 mice. The tumor-bearing foot was radiated with 30Gy in a single dose 7 days later. The left legs with growing tumors plus regional popliteal lymph nodes were amputated at 1,2,3,4 or 5mm primary tumor size and lymph node metastases documented. The primary melanoma was first visible 18 ± 3 (SD) days after inoculation in controls and 48 ± 5 days (SD) in radiated mice. After this radiation-induced regrowth delay primary tumor growth rate was parallel to that of control mice. Melanoma was present in the popliteal lymph nodes in 0, 18, 58, 50 and 82% of control mice and 0, 21, 47, 55 and 80% of mice with radiated tumor for respective primary tumor sizes 1-5 mm. Mice were sacrificed 18 days after amputation and pulmonary metastases counted. Pulmonary metastases were present in 0, 27, 17, 42 and 82% of control mice and 0, 18, 35, 47 and 80% of mice with radiated tumor. There is a direct correlation between increasing primary tumor size and the incidence of metastases ($p=<0.0001$, logistic regression analysis, for both lymph node and pulmonary metastases). Melanoma cells appear to have an unchanged metastatic potential after subcurative radiation in vivo, suggesting that there is no differential radiation sensitivity of cells with the metastatic phenotype.

Q 317 **ISOLATION AND VISUALIZATION OF MET-72 POSITIVE, METASTATIC VARIANTS PRESENT IN B16 MELANOMA TUMOR MASSES,** Nanette P. Parratto and Arthur K. Kimura, University of Florida, Gainesville, Florida 32610.
Metastatic variants of the B16 melanoma displaying high experimental metastatic potential have been shown to express high levels of a 72,000 dalton glycoprotein (Met-72) on their cell surface[1]. Monoclonal antibodies (MoAb) directed against the Met-72 determinant have been used in this study as immunohistochemical reagents on preparations of fresh B16 melanoma tumors and their metastases. These immunohistochemical analyses have utilized frozen sections, impression smears and cytospin preparations of fresh tumors harvested at various time points during tumor progression, to view the presence and location of Met-72 positive metastatic variants within tumor masses. Biotinylated anti-Met-72 MoAbs were reacted with freshly dissociated tumor cells from a B16 melanoma ovarian metastasis. These cells were then reacted with FITC-streptavidin and analyzed by flow cytometry. A discrete population of positively staining cells was detected and isolated by cell sorting techniques. Met-72 positive cells were then cloned and reanalyzed after several weeks of in vitro expansion and found to have high experimental metastatic potential to ovaries. Frozen sections of subcutaneous tumors and their metastases were analyzed by immunoperoxidase techniques. A consistent finding in these studies has been that the few tumor cells which showed high intensity of Met-72 staining were positioned on the invading front of B16 melanoma tumors. [1]Kimura, A.K. & Xiang, J. JNCI 76:1247-2154 (1986). This work was supported in part by NIH grant CA 40351.

Q 318 THE ROLE OF INTERFERON AND NK CELLS IN THE PREVENTION OF B16F10 PULMONARY METASTASES BY 3,6-BIS(2-PIPERIDINOETHOXY)ACRIDINE TRIHYDROCHLORIDE (CL 246,738), V. Ruszala-Mallon, J. Silva, A. Lumanglas, B. S. Wang and F. E. Durr, Lederle Laboratories, Pearl River, NY 10965.
CL 246,738 is an orally active small molecular weight synthetic immunomodulator previously shown to inhibit the development of tumor metastases. This study was done to determine the mechanism by which the compound functions as an anti-metastatic agent. Treated and untreated C57Bl/6 (B6) mice and B6 beige mice were injected I.V. with B16F10 melanoma cells and were sacrificed 3 weeks later to determine the number of tumor nodules in the lung. Treatment with CL 246,738 one or two days prior to injection of tumor cells significantly decreased the numbers of grossly visible pulmonary metastases and accelerated the clearance of IV-injected ^{125}IUDR-labeled tumor cells from the lungs. However, the anti-metastatic effect was dramatically reduced when B6 or beige mice were treated with anti-ASGM-1 antibody at the same time as drug, suggesting the importance of NK cells. Furthermore, when drug treatment was followed by I.V. injection of anti-IFN antibody both anti-metastatic and enhanced NK activities induced by the compound were diminished, indicating that IFN was an essential mediator. Therefore, it appears that the anti-metastatic effect of CL 246,738 is closely associated with its ability to induce IFN which in turn augments NK cell activity.

Q 319 Double antibody RIA in conjunction with immunoblotting for the specific detection of a serum breast tumor marker. Robyn Schecter, Pierre P. Major, McGill University, Montreal, P.Q. Canada H3G 1Y6.
We have identified the optimal antibodies for use in a double antibody RIA. Antibody MA9, adsorbed to the surface of PVC microtiter wells, is used to "capture" antigen present in patient sera. A second antibody, MA6, labeled with I-125 is then used to detect the presence of immobilized antigen. The assay is reproducible and stoichiometric over a wide range. Results show that approximately 90% of patients with metastatic breast cancer have detectable antigen and that the levels of antigen follow the course of disease showing an increase in antigen levels with progressing disease and a decrease in levels with progression of disease under treatment. In addition we have used this assay to detect circulating antigen in patients with primary breast cancer prior to mastectomy and have found approximately 40% of patients with elevated levels of circulating antigen. Sera of women with fibrocystic disease and benign breast tumors do not show any significant elevations. Some patient sera give RIA values which are in the upper range of normal values. To discriminate between higher RIA values of normal women and low positive values of patients, we immunoperoxidase stained Western blots of serum samples enriched for high molecular weight proteins by gel permeation chromatography. Using this technique we can see the positively staining bands in patient sera with low RIA values, while no such bands are seen in the sera of normal women with equivalent RIA values. Our approach of serum immunoblotting should contribute important information and may allow the development of a more specific assay than currently exists.

Q 320 ANTIINVASIVE EFFECT OF ESTRAMUSTINE IN VITRO, Guy A. Storme, Christian H. Dragonetti, Georges K. De Bruyne, Beryl Hartley-Asp, Marc M. Mareel, Oncologic Center-Radiotherapy, Vrije Universiteit Brussel ; Laboratory of Experimental Cancerology, Department of Radiotherapy and Nuclear Medecine, University Hospital R.U.G., Belgium.
Estramustine (EM) is mainly used to treat prostatic cancer. Recently it was shown that EM induced mitotic arrest which could be explained by its antimicrotubule effects. We wondered wheter EM shared the antiinvasive activity of microtubule inhibitors. Spheroids of mouse fibrosarcoma cells (MO4) or human prostatic tumor cells (Du145) were associated with precultured heart fragments (PHF). At a dose of 5.84 µg/ml of EM, the MO4 cells failed to invade the PHF. When the drug was washed out, the MO4 cells invaded the PHF and mitotic figures were observed. Estradiol (E) and nor-nitrogen mustard (NM) at respectively a dose of 3.765 and 2.17 µg/ml, didn't alter the invasion pattern. Immunocytochemical analysis of the microtubules showed a disturbed pattern of the cytoplasmic microtubules (CMT) and multipolar mitosis after 3 hours of incubation at 5.84 µg/ml of EM. After washing out the drug, a normal CMT and normal mitosis were observed. The same observations were done with regard to Du145 cells at a dose of 2 µg/ml EM. A dose of 0.1 µg/ml vinblastine (VLB) showed a disturbed CMT of MO4 cells. Combination of EM and VLB at the formentionned doses abolished completely the CMT indicating an additive effect of these drugs. However, when VLB at a dose of 1 µg/ml, which inhibited completely CMT, was washed out with EM 5.84 µg/ml, a disturbed CMT pattern reappeared. EM seems to be an interesting molecule since it interacts with microtubules in a way that is different from the colchicine-like drugs. Supported by a grant of ASLK and FGWO 3.0046.86.