CANCER CELL INVASION AND MOTILITY

Organizers: Garth L. Nicolson and Lance A. Liotta

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Poster Sessions

Metalloproteinase Gene Structure and Regulation

B1-001 SIGNAL TRANSDUCTION MECHANISMS CONTROLLING MATRIX METALLOPROTEINASE-1 (MMP-1) PRODUCTION IN NORMAL FIBROBLASTS AND IN A2058 MELANOMA CELLS. Teresa I. Mitchell*, Ian M. Clark# and Constance E. Brinckerhoff*, Dartmouth Medical School*, Hanover, NH 03755 and Addenbrooke Hospital#, Cambridge UK.

Collagenase (MMP-1) has the singular ability to degrade collagen types I, II and III at neutral pH, and production of this enzyme by tumor cells and neighboring normal fibroblasts contributes to the invasive potential of tumors. In normal fibroblasts, constitutive levels of MMP-1 are low, but can be stimulated by agents such as phorbol esters. In contrast, A2058 human melanoma cells show strikingly high constitutive levels of MMP-1 and can not be induced by phorbol. We used several kinase inhibitors, the PKC inhibitors Bisindolymaleimide (BIS) or Calphostin C (CAL), and the soluble tyrosine kinase inhibitor Herbimycin (HERB), to investigate the signal/transduction mechanisms responsible for phorbol inducibility in rabbit fibroblasts and for high constitutive expression in A2058 cells. Western blot analysis showed that a 24 hour treatment with BIS (10^{-6} and 10^{-7}M) or CAL (10^{-6}M) reduced MMP-1 levels in phorbol-stimulated fibroblasts but did not reduce MMP-1 levels in A2058 cells. In contrast, 24 hours exposure to HERB (10^{-6} and 10^{-7}M) lowered MMP-1 levels in tumor cells but not in PMA-treated fibroblasts. Results of Northern blots were similar, with CAL causing a 70% reduction in MMP-1 mRNA in PMA-treated fibroblasts and HERB giving an 85% and 65% decrease in MMP-1 mRNA in cells treated with 10^{-6} and 10^{-7}M, respectively. These data indicate a PKC-dependent component to phorbol-induced MMP-1 in fibroblasts, and a PKC-independent pathway in tumor cells that involves a soluble tyrosine kinase. None of the inhibitors was toxic to the cells at the concentrations used, as measured by incorporation of ^{3}H-leucine into protein.

Nuclear run-on assays with nuclei harvested at intervals over 24 hours from the A2058 cells showed continual transcription. This was accompanied by constitutive expression of c-jun mRNA and strong binding of nuclear extracts from A2058 cells to an oligonucleotide containing the AP-1 site at -77 of the rabbit collagenase promoter. Similar to complexes from fibroblasts, the DNA/protein complexes could be "super-shifted" specifically with antibodies to jun. These findings suggest constitutive transcription of the collagenase gene in A2058 cells, and implicate the AP-1 site in this transcriptional activation. We conclude that the AP-1 site is involved in transcriptional activation of the collagenase gene in both fibroblasts and tumor cells, but that the signal /transduction pathways leading to this transactivation differ in normal vs. tumor cells.

B1-002 MECHANISM OF CELL SURFACE ACTIVATION OF THE 72 KDA TYPE IV COLLAGENASE: ISOLATION OF THE ACTIVATED FORM OF THE MEMBRANE METALLOPROTEASE. Alex Y. Strongin, Ivan E. Collier, Gregory A. Bannikov, Barry L. Marmer, Gregory A. Grant, and Gregory I. Goldberg, Washington University School of Medicine, Department of Medicine, St. Louis, MO 63110

Metalloproteases are secreted by mammalian cells as zymogens and upon activation initiate tissue remodeling by proteolytic degradation of collagens and proteoglycans. Activation of the secreted proenzymes and interaction with their specific inhibitors TIMP-1 and TIMP-2 determine the net enzymatic activity in the extracellular space. We have previously demonstrated that 72T4Cl can be activated by a plasma membrane dependent mechanism, specific for this enzyme. Here we report purification of the membrane activator of 72T4Cl, which is a new metalloprotease (MT-MMP) that acts as a cell surface TIMP-2 receptor with Kd = $2.54X10^{-9}$M. The activator-TIMP-2 complex in turn acts as a receptor for 72T4Cl (Kd = $0.56x10^{-9}$M), binding to the carboxyl-end domain of the enzyme. Activation of 72T4Cl on the cell membrane provides a basic mechanism for spatially regulated extracellular proteolysis and presents a new target for prognosis, and treatment of metastatic disease.

The activator, purified as a tri-molecular complex of MT-MMP/TIMP-2/carboxyl-end domain of 72T4Cl, is itself an activated form of MT-MMP, posing the question: what is the mechanism of the activator's activation? The presence of an RRKR sequence just upstream of the amino terminus of the activated MT-MMP suggests that activation of 72T4Cl is the result of a cell surface proteolytic cascade that involves activation of MT-MMP by membrane associated plasmin and/or plasminogen activator which itself is activated upon binding to a specific receptor.

B1-003 MMP-2 AND MMP-9 TYPE IV COLLAGENASES, Karl Tryggvason[1], Paula Reponen[1], Carin Sandberg[2], Carine Munaut[1] and Irma Thesleff[2], [1]Biocenter Oulu and Department of Biochemistry, University of Oulu, [2]and Department of Pedodontics and Orthodontics, University of Helsinki, Finland.

The matrix metalloproteinases MMP-2 and MMP-9, also termed 72 kDa and 92 kDa type IV collagenases or gelatinases A and B, respectively, are structurally similar proteins, but they differ from interstitial collagenases and stromelysins in that both contain three internal repeats that resemble the gelatin binding domains of fibronectin. Additionally, MMP-9 contains a 48-residue segment of unknown function not present in MMP-2. Both enzymes cleave triple helical type IV collagen at a single site, they have some activity against other collagen types and also high activity against denatured collagen (gelatin). The two enzymes have been implicated in the turnover of basement membranes and in extracellular proteolysis during tumor invasion. We have demonstrated that during mouse development MMP-2 is, with the exception of epithelial cells of salivary gland, expressed in mesenchymal cells. Also, the activity is high in the embryo, but decreases after birth. MMP-2 is likely to have a major function for normal turnover of collagen of the extracellular matrix during tissue development, growth and tissue regeneration. The spatial expression of MMP-9 was shown to be completely different, as it is primarily observed in osteoclasts during bone formation in the mouse embryo and after birth. This enzyme may, in fact, have a major role for turnover of collagen in bone. Additionally, expression is observed in trophoblasts of the implanting embryo and in macrophages in tissue injury. The different temporal and spatial tissue distribution of expression is reflected in highly diverged gene regulation. The physiological substrates for MMP-2 and MMP-9 are unknown, but their location indicates that they act on collagenous proteins or their denatured fragments (gelatin) during matrix turnover.

In tumor invasion MMP-2 expression is usually greatly enhanced in fibroblasts surrounding tumor cells as shown by *in situ* hybridization while, surprisingly, the enzyme protein is mainly located in the tumor cells as judged by immunohistochemical methods. The explanation for this is unclear, but it indicates that MMP-2 is somehow bound to the tumor cells where it may exhibit its activity. The MMP-9 enzyme expression is also induced in many tumors. By *in situ* hybridization the activity is usually mainly observed in macrophages surrounding the invasion front, but it has sometimes also been localized to the tumor cells themselves.

In conclusion, MMP-2 and MMP-9 are closely related enzymes that probably have similar substrate activities, but have different temporal and spatial expression and, thus different developmental roles. Expression of both enzymes is increased in invasive tumors indicating their role in breakdown of matrix during tumor invasion.

Cancer Cell Invasion and Motility

Matrix Metalloproteinases and Tumor Invasion

B1-004 THE ROLE OF MATRILYSIN IN HUMAN PROSTATE TUMOR CELL INVASION, G. Tim Bowden[1], J. David Knox[1], William C. Powell[2], Dorothea C. von Bredow[1], Padma Sundareshan[1], Russell D. Klein[1], Jeffrey L. Boyd[1], Anne E. Cress[1], and Raymond B. Nagle[1], [1]University of Arizona Medical School, Tucson, AZ 85724, [2]Vanderbilt University School of Medicine, Nashville, TN 37232

Matrilysin, a member of the family of metalloproteinases, is known to be expressed in a variety of human tumors including invasive prostate tumors and in some types of normal tissue. Unlike other members of the family, it does not contain the carboxy-terminal hemopexin-like domain. Matrilysin is known to degrade a number of in vitro substrates including fibronectin, laminin, entactin, elastin, gelatin and proteoglycans. However, very little is known concerning the in vivo substrates for this protease. In this regard we have shown that purified matrilysin can degrade fibronectin fibrils produced by human fibroblasts. In addition, we identified specific fibronectin degradation fragments resulting from the proteolytic activity of matrilysin. We have shown that matrilysin is overexpressed focally in invasive primary prostate tumors at both the messenger RNA and protein levels. To determine whether matrilysin has a functional role in prostate tumor cell invasion, we expressed the protease in the tumorigenic but weakly invasive and non-metastatic human prostate tumor cell line, DU-145. DU-145 cells expressing matrilysin were significantly more invasive than vector-only transfected cell lines as assayed in an ectopic model of i.p. injection into severe combined immunodeficient mice. In this model we used diaphragm invasion as an end point. Since we did not observe metastasis and it has been reported that orthotopic administration of human tumor cells can result in metastasis, we repeated the comparison of the cell lines using orthotopic injection into the dorsolateral prostate of the SCID mouse. We found again no evidence of metastasis. Similar to what was observed in the diaphragm invasion model, the DU-145 cells expressing matrilysin showed increased local invasiveness. Finally we have begun to study the regulation of matrilysin gene expression in prostate tumor cell lines. We have found that the prostate tumor cell line, LNCap, expresses matrilysin protein and a low level of messenger RNA. Co-cultures of LNCap cells and prostate derived fibroblasts resulted in enhanced level of matrilysin protein in the conditioned medium. Preliminary experiments indicate that a paracrine factor secreted by the prostate fibroblasts stimulates matrilysin expression in the LNCap cells. Therefore, our data support a functional role for matrilysin in local prostate tumor cell invasion and evidence suggests that expression of the matrilysin gene in tumor cells may be regulated by factor(s) secreted by prostate stromal fibroblasts. (This work was supported in part by NIH Grants CA-56666, ES-O6694 and CA-23074r).

Degradative Enzyme Regulation and Invasion I

B1-005 REGULATION OF PROTEASE EXPRESSION BY PROTO/ONCOGENES, Ernst Lengyel[1], Rebecca Gum[1], Ed Murphy[1], Mien-Chie Hung[1], Dihua Yu[1], Balraj Singh[2], Claus Nerlov[3], Morten Johnsen[3], Michael Birrer[4] and Douglas Boyd[1]. [1]Departments of Tumor Biology and [2]Molecular Pathology, M.D. Anderson Cancer Center, Houston; [3] Institute of Microbiology, University of Copenhagen, Denmark; [4]National Cancer Institute, Bethesda.

Tumor cell invasion depends, at least in part, on proteolytic attack of the surrounding basement membrane/extracellular matrix. One of the hydrolases implicated in this process is the urokinase-type plasminogen activator (u-PA) which converts the inert zymogen plasminogen into the widely acting serine protease plasmin the latter which degrades several extracellular matrix components. In squamous cell carcinoma (SCC), u-PA is synthesized by the tumor cells. However, the molecular mechanisms responsible for the elevated expression of u-PA in this malignancy remain unclear. It appears that transcriptional activation of the u-PA gene, rather than gene amplification or increased mRNA stability, is responsible for the elevated level of this protease evident both *in vitro* and *in vivo*. Transient transfection assays employing both 5' deletion and mutated u-PA promoter CAT constructs indicated the importance of binding sites for AP-1 and PEA3/c-*ets* in a region of the promoter -1973 to -1885 bp upstream of the transcriptional start site. High u-PA-producing SCC cells possessed abundant AP-1 activity as evidenced by a strong activation of an AP-1-driven *tk* CAT construct. Moreover, u-PA promoter activity could be repressed by co-expression of a c-*jun* mutant (TAM-67), which lacks the transactivation domain, suggesting a role for c-*jun* in the overexpression of u-PA in this malignancy. Since the transcription factors which bind to AP-1 (c-*jun* and c-*jun/fos*) and PEA3 (c-*ets*) represent targets for proto/oncogenes involved in the signal transduction pathway, we undertook a study to determine the effect of a serine/threonine kinase- (v-*mos*) and a tyrosine kinase-(HER2/*neu*) encoding proto/oncogene in the regulation of u-PA expression in head and neck SCC. Transient expression of the serine/threonine kinase-encoding v-*mos* and a CAT reporter driven by the full length u-PA promoter in a squamous cell carcinoma cell line, UM-SCC-1, led to a dose-dependent increase in chloramphenicol acetylation. This effect was entirely attributed to the kinase activity of the v-*mos* since an expression vector mutated in this region of the oncogene did not stimulate CAT activity from the u-PA promoter. Stimulation of u-PA promoter activity by v-*mos* required binding sites for AP-1 -1967 and -1885 bp upstream of the u-PA transcriptional start site since mutation of these motifs abrogated the induction of u-PA promoter activity by the protooncogene. We also investigated the effect of the tyrosine kinase-encoding HER2/*neu*, which has been implicated in tumor metastasis, on u-PA expression. The stable expression of this proto/oncogene led to an induction of u-PA mRNA/protein in H460 lung cancer cells. This stimulation reflected, at least in part, increased u-PA promoter activity as shown in transient co-transfection assays. Utilization of 5' deletion and mutated u-PA promoter CAT constructs revealed that a PEA3/c-*ets* binding site at -1973 was required for the induction of u-PA expression by HER2/*neu*.

B1-006 TGF-ß FORMATION; MECHANISMS AND CONSEQUENCES, **Daniel B. Rifkin, New York University Medical Center, New York, NY 10016.**

TGF-ß, as normally found in tissues and media conditioned by cells, is composed of a high molecular weight complex in which the active growth factor remains noncovalently bound to its propeptide. This complex, sometimes known as small latent complex, is inactive and will not react with TGF-ß high affinity receptors. In addition a higher weight molecular complex exists that consists of the small latent complex plus a second gene product, the latent TGF-ß binding protein (LTBP), disulfide bonded to the propeptide. The large latent complex also does not interact with high affinity TGF-ß receptors. We have found that conversion of latent TGF-ß to TGF-ß requires a cell surface assemblage of proteases and binding factors. The required proteases are plasminogen activator and plasmin. The components of the larger latent complex that interact with the cell and/or matrix appear to be the LTBP and mannose-6-phosphate residues on the propeptide. In addition, the enzyme transglutaminase is necessary for activation as inhibitors of tissue transglutaminase block activation. These requirements govern TGF-ß formation in cocultures of vascular cells as well as homotypic cultures of either activated peritoneal macrophages or endothelial cells treated with agents such as retinoids. In the case of cocultures of endothelial and smooth muscle cells, the endothelial cells supply the proteolytic component, plasminogen activator, as well as the transglutaminase, whereas the smooth muscle cells provide binding sites for the latent TGF-ß.

Because the amounts of TGF-ß formed in these experiments are relatively low (20-50 pg/ml), we have developed a bioassay for TGF-ß that employs a chimeric cDNA comprising a region of the PAI-1 promoter fused to the luciferase gene. This construct was transfected into mink lung epithelial cells and permanent transfectants isolated. These transfectants, when exposed to TGF-ß, respond with increased expression of luciferase that can be quantitated automatically in a luminometer using 96 well dishes. This assay yields linear responses within a range of 5-200 picrograms of TGF-ß per ml. The response is reatively specific as basic fibroblast growth factor PDGF, EGF, and LPS have no effect on the assay.

We have recently begun to explore the regions of the LTBP required for activation. We have made expression vectors that include regions of the LTBP derived from the amino-terminus, the central region, and the carboxy-terminus of the protein fused to GST. When these peptides were tested in activation assays, the peptide representing the carboxy-terminal region blocked activation, whereas the peptides representing the amino-terminal or the central regions of the molecule had no effect. Antibodies prepared against each of these peptides were tested in activation assays for their ability to inhibit production of TGF-ß. The antibody against the caroxyl-terminal region blocked activation, whereas antibodies against the amino-terminal and central regions of LTBP were inactive. The antibodies and peptides to LTBP inhibitied TGF-ß generation in both cocultures of endothelial and smooth muscle cells and activated mouse peritoneal macrophages.

Degradative Enzyme Regulation and Invasion II

B1-007 BIOLOGICAL AND CLINICAL SIGNIFICANCE OF CATHEPSIN D IN BREAST CANCER METASTASES, Henri Rochefort, Patrick Augereau, Jean-Paul Brouillet, Emmanuelle Liaudet, Philippe Montcourrier, and Marcel Garcia, Unité Hormones et Cancer (U 148) INSERM, et Faculté de Médecine, Université de Montpellier 1, 60 rue de Navacelles, 34090 Montpellier, France.

Human breast cancer is known to develop early metastasis and to be stimulated by estrogens and inhibited by antiestrogens. Among the estrogen regulated proteins, cathepsin D (cath D), a lysosomal aspartyl protease, is of particular interest since its cytosolic concentration in primary tumor is associated with the risk of developing metastasis in several independent retrospective and prospective clinical studies. Immunohistochemical staining and *in situ* hybridization strongly suggest that cancer cells rather than macrophages or fibroblasts are responsible for this overexpression. Overexpression of stably transfected human cathepsin D in tumor cells also increased their metastatic potential in the nude mice. We investigated the mechanism underlying this facilitating effect of cath D on metastasis. Cath D maturation in acidic compartments appears essential for cath D to be active in metastatic process. Recent results suggest that cath D overexpression, by facilitating intracellular digestion of phagocytosed and endocytosed extracellular material, stimulates invasion of surrounding tissue by cancer cells. We will also report on the mechanism of cath D gene regulation by estrogen and growth factors including characterisation of an ERE and other regulatory sequences in the proximal promoter region. We propose that this protease acting in concert with others, facilitates tissue remodeling and growth of micrometastasis in breast cancer. Better understanding of the mechanism of action of cath D in the metastatic process might help to develop new therapeutic agents aiming to prevent clinical metastases.

B1-008 REGULATION OF EXPRESSION OF CATHEPSIN B IN HUMAN CANCERS, Bonnie F. Sloane[1], Isabelle M. Berquin[1], Mansoureh Sameni[1], Nancy A. Day[1], Michael Emmert-Buck[2], and Elias Campo[3], [1]Wayne State University, Detroit, MI 48201, [2]National Cancer Institute, Bethesda and [3]University of Barcelona, Spain.

The cells at the invasive edge of malignant tumors express the highest levels of staining for cathepsin B. These cells also exhibit the highest activity of cathepsin B, thus supporting a functional role for cathepsin B in tumor invasion. Cathepsin B may facilitate invasion directly by degrading extracellular matrix barriers like the basement membrane or indirectly by activating other proteases. Activity of cathepsin B is regulated at multiple levels: transcription, post-transcriptional processing, translation and glycosylation, maturation and trafficking, and inhibition. Our immunohistochemical studies of cathepsin B in human colon carcinomas show a striking correlation between increased staining for cathepsin B and progression as well as shortened patient survival. In human colon carcinomas, bladder carcinomas and gliomas, we have observed alterations in the localization of cathepsin B similar to those we reported earlier in tumor model systems. Levels of cathepsin B mRNA, protein and activity correlate with *in vivo* invasiveness/progression of the gliomas and colon carcinomas. Nevertheless, regulation of cathepsin B at the transcriptional and post-transcriptional levels is still poorly understood. Although putative promoter regions have characteristics of housekeeping-type promoters, cathepsin B mRNA expression varies depending on the cell type and state of differentiation. We have evidence that more than one promoter could direct expression of human cathepsin B. Multiple transcript species have been detected, resulting from alternative splicing in the 5'- and 3'-untranslated regions, and possibly the use of alternative promoter regions. The existence of transcript variants indicates a potential for post-transcriptional control of expression. In support of this, *ras*-transformation of MCF-10A human breast epithelial cells results in an increase in protein levels without a concomitant increase in mRNA levels. Cathepsin B mRNA species with distinct 5'-or 3'-untranslated regions may differ in their stability and translatability. Variations in the coding region may also alter the properties of cathepsin B. Two groups have observed transcript species that would encode a truncated protein, lacking the prepeptide and about half of the propeptide. This truncated protein, if synthesized in cells, would be expected to be cytosolic. We have established that alterations in the trafficking of cathepsin B seen in malignant tumor cells occur at early stages of progression of the MCF-10A human breast epithelial cells from preneoplastic to neoplastic, i.e., at the point of transition between the pre-neoplastic and neoplastic state and coincident with acquisition of the ability to invade *in vitro*. Cathepsin B is also present on the surface of human breast carcinoma cells as well as other human tumors. Having cathepsin B at the cell surface may facilitate local degradation of extracellular matrix by cathepsin B and/or by proteases activated by cathepsin B, e.g., prostromelysin. As the trafficking of cathepsin B and other vesicular proteases to the cell surface appears be a mechanism common to cells that engage in local degradative and invasive processes, defining the processes that regulate trafficking of cathepsin B may be critical to our understanding of tumor invasion.

Models of Tumor Invasion and Angiogenesis

B1-009 ROLE OF AMINOPEPTIDASE N/CD13 IN INVASION AND METASTASIS OF HUMAN MELANOMA CELLS, Motowo Nakajima[1], Hideji Fujii[1], Ikuo Saiki[2], Junya Yoneda[3], Ichiro Azuma[3], and Takashi Tsuruo[1]. [1]Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo 113, [2]Research Institute for Wakan-yaku, Toyama Medical and Pharmaceutical University, Toyama 930-01 and [3]Institute of Immunological Science, Hokkaido University, Sapporo 060, Japan

Aminopeptidase N/CD13 is a cell surface metalloprotease with a transmembrane domain. We have previously shown that aminopeptidase inhibitors suppressed the degradation and invasion of extracellular matrix (ECM) by metastatic melanoma cells. We have also found a localization of aminopeptidase N/CD13 at the invasion edge of melanoma cells. The association of aminopeptidase N expression with malignant conversion of melanocytes has recently been reported. Thus, we further examined a role of aminopeptidase N/CD13 in invasion and metastasis of human melanoma cells. We found a good correlation of aminopeptidase N activity and invasiveness of several human melanoma cell lines. Human A375M melanoma cells showed moderate levels of aminopetidase N activity. We transfected A375M melanoma cells with eukaryotic expression plasmid vectors containing the full length cDNA of aminopeptidase N/CD13. The transfectants expressing higher levels of aminopeptidase N/CD13 showed higher activities to degrade type IV collagen and invade ECM than the parental and control vector-transfected cells. Furthermore, a significant augmentation of lung metastatic potential was demonstrated by the aminopeptidase N/CD13-transfectants in nude mice. The results suggest that aminopeptidase N/CD13 plays an important role in melanoma cell invasion and metastasis.

B1-010 ANGIOGENESIS: A BALANCE BETWEEN POSITIVE AND NEGATIVE REGULATORS

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Angiogenesis is an absolute requirement for the growth of normal and neoplastic tissues. Capillary sprout formation is characterized by basement membrane degradation and endothelial cell migration, processes which are dependent on a cohort of proteases and protease inhibitors produced by both endothelial and non-endothelial cells. The activity of cytokines involved in the control of angiogenesis is likewise regulated by extracellular proteolysis. The plasminogen activator (PA)-plasmin system has been extensively studied in these processes. Traditionally, urokinase-type PA (uPA) has been implicated in extravascular matrix degradation in situations of tissue remodelling and cell migration, while tissue-type PA (tPA) has been implicated in intravascular thrombolysis. In vitro studies will be described which have led to the notion that the PA-plasmin system is important in angiogenesis, and that normal capillary morphogenesis is dependent on a protease-antiprotease equilibrium. The advent of uPA-, tPA- and PAI-1-deficient mice, in which developmental and physiological angiogenesis appear to occur normally, has led to a re-evaluation of this dogma. This will be discussed in the context of descriptive in vivo studies which demonstrate uPA and PAI-1 in endothelial cells during angiogenesis in vivo, and of studies using the polyoma virus middle-T oncogene to induce endothelial cell tumors in uPA-, tPA- and PAI-1 deficient mice.

Endothelial cell activation status is determined by a balance between positive and negative endogenous regulators. In resting endothelium, quiescence is maintained by the dominance of negative regulators; angiogenesis is induced when balance is tipped in favour of positive regulators. In vitro findings will be described which support the notion that the activity of angiogenesis-regulating cytokines is contextual, in that it depends on the presence and concentration of other cytokines in the pericellular environment of the responding endothelial cell. Finally, experiments will be described which have led to the identification of genistein and 2-methoxyestradiol as negative regulators of angiogenesis, and therefore as potentially novel therapeutic agents for the treatment of angiogenesis-dependent diseases.

B1-011 INDUCTION OF PATTERNS OF PROTEASE EXPRESSION AND THE ROLE OF PROTEASES AND PROTEASE INHIBITORS IN METASTASIS.

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Patterns of gene expressions in metastatic cells indicate a redundancy in transformation pathways leading to metastasis as well as angiogenesis. We show a clear example of apparent redundancy in a study of how *ras* oncogenes generate the metastatic phenotype, utilizing NIH 3T3 cells transformed by different Ras isoforms [Zhang and Schultz, *Cancer Research 52*, 6682 (1992)]. One *ras*-transformed phenotype, designated $ras^{uPA+/CL-}$, shows high constitutive expression of urokinase plasminogen activator (uPA) and/or phorbol ester (TPA) induction of uPA expression. The second phenotype, designated $ras^{CL+/uPA-}$, shows neither high constitutive expression nor TPA induction of uPA. However, this later phenotype shows high constitutive expression and/or TPA induction of the cysteine protease cathepsin L (CL). Using antisense oligonucleotides we have selectively inhibited either uPA expression or CL expression and shown that uPA facilitates experimental metastasis in the first phenotype, but not the second. In contrast, CL facilitates experimental metastasis in the second phenotype and not the first. Investigation of the pathways downstream from Ras in the two phenotypes shows that different signal transduction pathways have been activated. Heterologous CAT expression vectors with the uPA upstream region from -2568 through -2182 from the start of transcription show this region of the promoter to be responsible for the 6-fold constitutively higher expression of uPA in the EJ/vHa-transformed cells of phenotype $ras^{uPA+/CL-}$. This region does not have enhancer activity in the alternative $ras^{CL+/uPA-}$ phenotype. A gel retardation assay of this upstream sequence shows a retarded protein-DNA band in EJ/vHa-*ras* transformed cells but not in untransformed NIH 3T3 cells or in $RAS1^{Leu}$del-transformed cells of phenotype $ras^{CL+/uPA-}$. Investigation of the Raf, MAPK, Jun/Fos signal transduction molecules downstream from Ras show points of divergence in the two *ras*-transformed phenotypes resulting in the selective phosphorylation of c-Jun and c-Fos in the EJ/vHa-*ras*-transformed cells (phenotype $ras^{uPA+/CL-}$). In the $RAS1^{Leu}$del-transformed cells alternative pathways inhibit uPA expression and upregulate CL expression.

Redundancy may also result from the over-expression of a metastatic promoting factor which obviates the requirement for another factor usually required for invasion and metastasis. This is shown by the lack of a requirement for uPA binding to cell surface receptors in the metastasis of murine cells transfected with the human uPA gene and expressing high amounts of uPA activity. Regulation of protease activity through either proteases or their inhibitors also demonstrate a redundancy in the regulation of metastasis. These later mechanisms are regulated by alternate signal transduction mechanisms that have identical functional endpoints. Therapeutic interventions need to take into account biological redundancy.

Cell Motility Mechanisms

B1-012 TRACING THE SIGNAL TRANSDUCTION PATHWAY FROM THE THROMBIN RECEPTOR TO ACTIN FILAMENT ENDS IN PLATELETS.

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Ligation of the thrombin receptor causes resting, discoid platelets to change shape in a reaction driven by the massive assembly of monomeric actin into filaments. Filament assembly starts when barbed filament ends, previously blocked with capping proteins in the resting cell, expose. These ends have higher affinities for actin subunits than β4-thymosin, the major actin subunit sequestering protein. The cytoplasmic components and signals that lead to filament uncapping have been studied in n-octyl-glucopyranoside permeabilized resting platelets that retain their responsiveness to the thrombin receptor activating peptide, TRAP, and have the same dose response to TRAP as do intact cells. Using permeabilized cells, the following pathway to filament ends has been dissected:

TRAP--> GTP-->*rac*--> $PI_{4,5}P_2$--> dissociation of capping proteins from actin filament ends.

The following information establishes this pathway: 1) Addition of GTPγS to permeabilized cells stimulates filament uncapping without requiring receptor ligation, while the addition of GDPβS inhibits uncapping in response to TRAP. 2) Addition of the constitutively active recombinant g-protein *rac* or micelles of $PI_{4,5}P_2$ overcomes the GDPβS inhibition. Recombinant *rho* protein was inactive. 3) All filament uncapping, however, is inhibited by 10-mer peptide that binds $PI_{4,5}P_2$ with high affinity. Control peptides of similar size and charge have no effect on uncapping. 4) The effect of this peptide can, however, be specifically overcome by exogenous $PI_{4,5}P_2$. Treatment of permeabilized platelets with TRAP or *racV* leads to synthesis of $PI_{4,5}P_2$. $PI_{3,4}P_2$ and $PI_{3,4,5}P_3$ are produced, but are unnecessary, since inhibition of PI-3 kinase has no effect on the ability of cells to repond to TRAP by assembling actin filaments. Likely targets of $PI_{4,5}P_2$ at platelet filament ends are gelsolin and capZ.

B1-013 INVASIVE TUMORS OF *DROSOPHILA* AS A MODEL OF METASTASIS, Elisa Woodhouse, Evelyn Hersperger, and Allen Shearn, Department of Biology, The Johns Hopkins University, Baltimore, MD. 21218.

Mutations in the *Drosophila* tumor suppressor gene *lethal giant larvae* cause loss of normal tissue structure, loss of capacity to differentiate and invasiveness of imaginal neuroblasts. Six additional *Drosophila* tumor suppressor genes have been identified by mutations which cause overgrowth of larval brains. Using cell-autonomous markers the invasive potential of neuroblastomas caused by mutations in each of these genes is being defined by two components: growth of primary tumors following transplantation into female sterile hosts and invasiveness of tumor cells following transplantation into normal hosts. Invasiveness is evaluated in terms of the range of host tissues invaded and the number of secondary tumors within each invaded tissue. The growth dynamics suggest that only a small fraction of the cells in transplanted mutant brains proliferate and give rise to secondary tumors and that tumor cells are distributed throughout the brains of *lethal giant larvae* but only in the optic lobes of *disc large* brains. The metastatic phenotype includes loss of normal tissue integrity and increased proteolysis of the extracellular matrix. Either or both of these processes can be mediated by gelatinases. Preliminary work has led to the detection of three *Drosophila* proteins with gelatinase activity and demonstrated increased accumulation of one of these three in brains dissected from *lethal giant larvae* and *discs large* mutants and in invasive neuroblastomas derived from such mutant brains. This gelatinase is a 49kDa protein with a pI of 7.2 that cross-reacts with antibody directed against human gelatinase A. The amount of gelatinase 7.2 accumulated in brains from *lethal giant larvae* and *discs large* mutant larvae is similar. So, the more widespread distribution of tumor cells in the brains of *lethal giant larvae* compared to *discs large* brains can not be due to differences in accumulation of this gelatinase.

Neuroblastomas caused by mutations in the *brain tumor* gene only invade organs near the site of transplantation whereas neuroblastomas caused by mutations in the *lethal giant larvae* and the *discs large* gene invade nearby as well as distant organs. Despite the open circulatory system of *Drosophila*, this result suggests that tumor cells are not distributed passively but rather that active motility plays a role in the wide range of tissues invaded by neuroblastomas caused by mutations in the *lethal giant larvae* and the *discs large* gene.

Cell Chemotaxis and Signaling

B1-014 SENSORY TRANSDUCTION IN *DICTYOSTELIUM*, Peter N. Devreotes, Pamela Lilly, Dale Hereld, Michael Caterina, Ji-Yun Kim, Mei-Yu Chen, Jacqueline Milne, Robert Insall, Carole Parent, Brenda Blacklock, and Zhan Xiao, Johns Hopkins Sch. Med., Baltimore, MD.

The G-protein coupled signal transduction pathways initiated by activation of the chemoattractant receptors cAR1-cAR4 are essential for chemotaxis, cell-cell signaling and gene expression during the developmental program of *Dictyostelium*. We have exploited this property to carry out random mutagenesis of cAR1, the α- and β-subunits of G2, and the adenylyl cyclase ACA. To map the high affinity cAMP binding domain of cAR1, we generated a series of chimeras between cAR1 and cAR2, which has a much lower affinity for cAMP. The cAMP binding properties and the EC_{50} for agonist-induced phosphorylation of these chimeras suggests that the major determinant of cAMP affinity is due to 5 amino acid differences within the second extracellular loop. To explore the domains in cAR1 required for its activation, we randomly mutagenized the third intracellular loop (24 amino acids) as well as the entire coding sequence and selected mutants by phenotypic rescue. We found a series of activation mutants that displayed wild-type affinity, but with impaired ability to induce G protein-dependent and -independent pathways, as well as mutants resembling cAR2, which has low affinity but can be fully activated at high agonist concentrations. To investigate agonist-induced phosphorylation of cAR1 we used a collection of cAR1 mutants that lack various combinations of 18 series within its C-terminal cytoplasmic domain. Cluster 1 (comprised of serines 299, 302, 303, 304, and 308), and, to a lesser extent, cluster 2 (series 324, 325, and 331) are the principal sites of phosphorylation. Mutants lacking all phosphorylation sites were severely impaired in cAMP-induced loss of ligand binding, but, to our surprise, the cAMP-induced cAMP secretory response of cells expressing stops normally. Thus, it appears that a mechanism other than receptor phosphorylation leads to desensitization.

In addition, we have used insertional mutagenesis to isolate novel genes in G-protein linked signaling pathways. Adenylyl cyclase in *Dictyostelium*, as in higher eukaryotes, is activated through a receptor/G protein pathway. Insertional mutagenesis into a gene designated *dagA* resulted in cells that cannot activate adenylyl cyclase, but have otherwise normal responses to exogenous cAMP. Neither cAMP treatment of intact cells nor GTPγS treatment of lysates stimulates adenylyl cyclase activity in *dagA* mutants. We show that *dagA* is the structural gene for CRAC, a cytosolic protein that activates adenylyl cyclase, and contains a pleckstrin homology domain. We hypothesize that CRAC acts to connect free G protein βγ subunits to adenylyl cyclase activation. If so, it may be the first member of an important class of coupling proteins.

B1-015 MUSCARINIC RECEPTOR TRANSFECTED CELL LINES AS MODELS FOR SIGNAL TRANSDUCTION-DEPENDENT TUMOR FORMATION AND SUPPRESSION, Christian Felder[1], Dafna Lahat[1], Sharon Savage[2], Alice Ma[1], Eileen Briley[1], Elise Kohn[3]. [1]Laboratory of Cell Biology, NIMH, [2]HHMI-NIH Medical Scholars Program, and [3]Laboratory of Pathology, NCI, Bethesda, MD.

Cell growth and differentiation is regulated through the activation of receptor proteins by cytokines, growth factors, and hormones and the propagation of their signals to the cell interior. The role of signal transduction in oncogene-mediated tumorigenesis has been well established and it has been shown that most oncogenes code for proteins involved in signalling cascades. More recently, G protein-coupled receptors have been shown to play a role in tumorigenesis and tumor suppression, but the signal transduction pathways involved have not been established. Ectopic expression of serotonin (1) muscarinic acetylcholine (2) or alpha-adrenergic receptor (3) cDNA in NIH 3T3 cells resulted in agonist dependent progression to a malignant phenotype in vitro and in vivo. G protein-coupled receptors transmit their signals through G protein activation and subsequent stimulation of effector enzymes or ion channels. We have demonstrated muscarinic agonist-dependent inhibition of the tumorigenic phenotype of CHO cells transfected with and expressing the m1, m3, and m5 receptor subtypes, but not the m2 and m4 receptor subtypes (4). Phenotypic regression from a stellate to fibroblastic morphology was used as a marker of carbachol- (muscarinic receptor agonist) mediated tumor suppression. The carbachol-induced morphology change was complete by 5 and 6 hours of treatment and correlated with a reduction in carbachol-stimulated calcium influx. The dose response of carbachol-stimulated morphology change correlated with carbachol-mediated calcium influx but not with activation of phospholipases A2, C, D, or adenylate cyclase. Calcium influx was essential for muscarinic receptor-dependent activation of phospholipases A2, Cγ, D, and tyrosine kinase. Actinomycin D (1 uM) or cyclohexamide (1 ug/ml) completely blocked the carbachol-induced morphology change indicating a requirement for protein synthesis. Time dependent addition of actinomycin D indicated a molecular commitment towards the morphology change after 3 hour treatment with carbachol. Our results suggest that m1, m3, or m5 receptor-activated calcium influx through receptor-operated calcium channels play a central role in the suppression of CHO cell proliferation, yet the subsequent signalling pathways leading to transcription regulation are not known. Receptor transfected cells may provide an useful model system in which to investigate signal transduction-dependent cell proliferation or tumor suppression.

1. Julius D, Livelli TJ, Jessell TM, and Axel R (1989) Science 244, 1057-1062.
2. Gutkind JS, Novotny EA, Brann MB, and Robbins KC (1991) Proc Natl Acad Sci USA, 88, 4703-4707.
3. Allen LF, Lefkowitz RJ, Caron MG, and Cotecchia S. (1991) Proc Natl Acad Sci USA 88, 11354-11358.
4. Felder CC, MacArthur L, Ma AL, Gusovsky F, and Kohn EC (1993) Proc Natl Acad Sci USA 90, 1706-1710.

B1-016 THE MTS1 GENE IN METASTASIS AND MOTILITY, Heide L. Ford[1], Raka Chakravarty[1], Mohammed Salim[1], Debbie Silver[2], Viqar Aluiddin[1], Jim Sellers[2], and Sayeeda Zain[1], [1]University of Rochester Department of Biochemistry, Rochester, NY 14642, [2]Laboratory of Molecular Cardiology, Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892.

The *mts1* gene is overexpressed in metastatic cells as compared to their nonmetastatic counterparts, and codes for a 101 amino acid protein belonging to the S100 subfamily of small Ca^{++}-binding proteins. Mts1 is expressed in normal mouse tissue types such as activated macrophages, T-lymphocytes, and trophoblast cells; all which exhibit the ability to be motile. Recent studies have demonstrated an induction of the metastatic phenotype upon transfection with *mts1*, providing direct evidence for the involvement of Mts1 in the process of metastasis.

We have taken two approaches to determine the role Mts1 plays in normal tissues as well as in metastatic cells: (1) Mts1 function has been assessed in mouse mammary adenocarcinoma cells (CSML-0 and CSML-100) and (2) Proteins that interact with the Mts1 protein have been identified. First, nonmetastatic CSML-0 cells, which do not express *mts1*, and metastatic CSML-100 cells, which express *mts1*, were characterized with respect to markers of metastasis, their ability to be motile and invasive *in vitro*, and their ability to be metastatic *in vivo*. CSML-100 cells appear to be more motile, invasive, and metastatic than CSML-0 cells. The cells were then transfected with constructs expressing antisense *mts1* or sense *mts1*, respectively, and the role of Mts1 in the processes of motility, invasion, and metastasis was assessed.

The second approach to elucidating the biological role of Mts1 involves detecting proteins which interact with the Mts1 protein. Utilizing several different *in vitro* methods to detect protein-protein interactions, nonmuscle myosin II was found to interact with Mts1 in the presence of 0.1mM $CaCl_2$. The effect of Mts1 binding on myosin activity will be discussed.

Tumor Cell Motility and Autocrine Motility Factors

B1-017 AMF AND HOST-TUMOR CELL INTERACTIONS IN INVASION AND METASTASIS, Mustafa Kh. Dabbous[1], Lena Haney[1], and Garth L. Nicolson[2], [1]The University of Tennessee, Memphis, TN 38163 and [2]The University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030.

Tumor invasion and metastatic spread reflect a tissue phenomenon in which cellular interactions between tumor cells and stromal fibroblasts play a pivotal role in the cellular mechanisms which underlie host tissue degradation and tumor cell motility. Existing evidence suggests that the enhanced release of collagenolytic enzymes by tumor cells and stromal fibroblasts, facilitate tumor invasion of the connective tissue stroma and basement membranes. Using rat 13762NF mammary adenocarcinoma, we have demonstrated that tumor cells produce their own metalloproteinases, as well as stimulate the production of higher levels of collagenolytic enzymes by tumor-associated stromal fibroblasts (TAF). The stimulation is specific, and varies with the metastatic potential of the invading tumor cells, and the presence of select subpopulations of responsive fibroblasts. Fibroblasts appear to be selective and exhibit clonal differences in the collagenolytic response to tumor-derived soluble factors and cytokines. The stimulatory activity, a protein, P_{54} with apparent mol. weight, Mr=54kDa, isolated and purified from media conditioned by the highly metastatic clone MTLn3, has a dual function; a paracrine fibroblast collagenase-stimulating function; and an autocrine motility-promoting function stimulating both the directed and random motility of Ln3 cells. Although, media conditioned by the low metastatic variant MTLn2 (Ln2-CM) contained cross-reacting protein component, Mr=54kD, they failed, however, to stimulate the motility of Ln2 or Ln3 cells to any significant extent. The lack of chemotactic activity in either Ln2-CM or co-cultures of Ln2 and Ln3 cells, suggested the presence of inhibitory activity in Ln2-CM. The chemotactic activity of Ln3CM was inhibited by the addition of Ln2 CM at 1:1 ratio. While Ln2 suppressed the chemotactic response of Ln3 cells, it stimulated the release of collagenolytic activity in co-cultures of the two cell clones. This suggests that clonal interactions within the tumor mass may contribute to the invasive growth, however, at the tumor periphery, stromal response may play a key role in matrix degradation and, in the initial tumor cell dispersion, thus, facilitating their metastatic spread. Tumor-associated fibroblasts (TAF) appear to represent a select fibroblast subpopulation, more responsive to tumor-derived signals. Lung metastasis clone MT Ln3, is more responsive to the chemotactic activity of media conditioned by TAF than to that of normal syngeneic fibroblasts (NRF-CM). Furthermore, the chemotactic response of the metastatic tumor cells appear to be selective and tissue-specific with maximum response to select stromal fibroblast clones derived from the organ of colonization. The production of migration-stimulating factor (MSF) and SF/HGF by stromal fibroblasts has been previously reported by several laboratories. Preliminary work from our laboratory suggest the presence of additional stromal cell-derived motility-promoting factors. Stromal influence on tumor cell invasion and motility may play a significant role in cancer progression.

B1-018 AUTOTAXIN, A NOVEL TUMOR CELL MOTILITY-STIMULATING PROTEIN, Mary L. Stracke and Lance A. Liotta, National Institutes of Health, National Cancer Institute, Laboratory of Pathology, Bethesda, MD 20892 USA

The human melanoma cell line, A2058, has been demonstrated to respond to a number of proteins in a motile fashion. These motility-stimulating proteins include the insulin-like growth factors, extracellular matrix proteins and a recently purified autocrine motility factor which we have termed autotaxin (ATX). ATX is a 125 kDa glycoprotein which elicits chemotactic and chemokinetic responses at picomolar to nanomolar concentrations. We utilized affinity-purified anti-peptide antibodies to the ATX peptide, ATX-102, to screen an A2058 cDNA expression library made in λ gt11. Positive clones were sequenced to obtain a partial cDNA clone of ATX which contained 1084 bases, including the polyadenylated tail, the AATAAA motif, and a 628 base pair open reading frame coding for 209 amino acids. This clone was extended by utilizing reverse trascriptase reactions on total cellular RNA followed by PCR amplification. The isolated cDNA clone contained 3251 base pairs, and the mRNA message size was approximately 3.3 kb. The deduced amino acid sequence of autotaxin matched 30 previously sequenced peptides and comprised a protein of 915 amino acids. Database analysis of the ATX sequence revealed a 45% amino acid identity (including 30 out of its 33 cysteines) with PC-1, a type I phosphodiesterase/pyrophosphatase expressed on the surface of activated B cells and plasma cells. ATX appears to define a new class of ectokinases which could play an important regulatory role in tumor cell motility.

Paracrine Motility Factors

B1-019 **ORGAN MICROVESSEL ENDOTHELIAL CELLS: SECRETION OF ORGAN-SPECIFIC MOTILITY AND GROWTH FACTORS,** Garth L. Nicolson, Hironao Wakabayashi, Jun-ichi Hamada[2]. Libin Jia and Philip G. Cavanaugh, Department of Tumor Biology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030 and [2]Laboratory of Cell Biology, Cancer Institute, Hokkaido University School of Medicine, Sapporo 060, Japan

We have been investigating the role of organ-derived microvessel endothelial cells in the organ preference of metastasis. Organ-derived microvessel endothelial cells in short-term culture express common and unique cell surface molecules and secrete motility factors and growth factors that differentially stimulate the movement and growth of organ-preferring malignant cells [1]. In addition, metastatic cells secrete angiogenesis factors (motility and growth factors) that stimulate microvessel endothelial cells in a process termed reciprocal stimulation [2]. For example, soluble factors released from mouse hepatic sinusoidal endothelial (HSE) and lung microvessel (LE) endothelial cells strongly stimulated the migration and growth of liver-colonizing and lung-colonizing cells, respectively. A major HSE motility factor for liver-metastatic cells has been isolated, partially sequenced and shown to be a proteolytic fragment of complement component C3b [3], and different motility factors have been isolated from LE and brain microvessel endothelial cells [4]. One of the LE motility factors has been purified and sequenced and shown to be macrophage chemotactic protein-1. Brain-derived endothelial cells also secrete motility factors that are capable of stimulating the chemotactic migration of brain-metastatic tumor cells, and these factors are different biochemically from the lung and liver endothelial cell-derived factors [5]. These organ endothelial cells also secrete tumor cell mitogens that differentially stimulate the growth of organ-preferring metastatic cells. One of these is related to transferrin (Tf), an important growth factor for metastatic cells [6]. We have cloned three different genes encoding Tf-like molecules that vary in homology (70-90%) with Tf [7]. Endothelial cells secrete Tf-like molecules in an organ-specific fashion (brain > lung > liver). Removal of Tf and Tf-like molecules does not completely inhibit mitogenic activity, suggesting that multiple tumor cell growth factors are secreted by the organ endothelial cells. Metastatic cells express Tf receptors in relation to their organ preference (brain > lung > liver) [6]. We are isolating and identifying the Tf-like and non-Tf tumor cell mitogens made by organ-derived microvessel endothelial cells. [1] Nicolson, G.L., Menter, D., Herrmann, J., Cavanaugh, P., Jia, L.-B., Hamada, J., Yun, Z. and Marchetti, D. *Crit. Rev. Oncogenesis,* in press, 1994. [2] Nicolson, G.L. *Exp. Cell Res.* 204: 171-180, 1993. [3] Hamada, J.-I., Cavanaugh, P.G. and Nicolson, G.L. *Cancer Res.* 53: 4418-4423, 1993. [4] Wakabayashi, H., Cavanaugh, P.G. and Nicolson, G.L. *Br. J. Cancer* in press (1994). [5] Hamada, J., Nicolson, G. L., Hosokawa, M. and Takeichi, N. *Clin. Exp. Metastasis* 12: 53, 1994. [6] Inoue, T., Cavanaugh, P.G. and Nicolson, G.L. *J. Cell. Physiol.* 156: 212-217, 1993. [7] Jia, L., Cavanaugh, P.G. and Nicolson, G.L. *Clin. Exp. Metastasis* 12: 21, 1994.

New Therapies and Diagnostic Applications

B1-020 PROSTATIC CANCER CELL MOTILITY AND METASTASIS, James L. Mohler[1], Alan W. Partin[2], Yousuf Sharief[1], Xuan-Zhi Zhang[1], William B. Coleman[3], Donald S. Coffey[2], and Gary J. Smith[3], [1]Division of Urology, Department of Surgery, [3]Department of Pathology and the UNC Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599 and [2]Brady Urological Institute, The Johns Hopkins University, Baltimore.

Metastases are clinically important because they prevent neoplasm ablation by operation or radiotherapy. Common to many steps of the metastatic process is a requirement for motility that cannot be appreciated by standard pathologic examination of fixed, dead tissues. The Dunning R-3327 rat prostatic adenocarcinoma model provides sublines whose biological behavior is not predictable by histology or numerous biochemical or morphological techniques. Visual grading of time-lapse videomicroscopic images distinguished in 96% of cases sublines of high and low metastatic capability when membrane ruffling, pseudopodal extension and retraction and cellular translation of single cells were analyzed. When prospectively tested in 11 Dunning sublines, highly metastatic cells were detected with a 94% sensitivity and 50% specificity. A more quantitative analysis of cancer cell motility was developed using complex fast fourier analysis. The motility of Dunning sublines tested was enhanced by application of autocrine motility factor or serum-free media conditioned by the highly motile, highly metastatic MAT-LyLu subline. However, of greater interest was the reduction in motility of MAT-LyLu cells by media conditioned by the non-motile, nonmetastatic G subline. This activity was heat and protease labile and proved by dialysis due to non-reduced 50-100 kD proteins. G-conditioned serum-free media was separated into 100 fractions by DEAE-cellulose chromatography. The motility inhibitory activity of G-conditioned media was localized to column fractions 51-70 that contained 18% of the applied protein and only 6.5% of the proteins secreted by the G cells. Analysis of pooled fractions 51-60 and 61-70 by 2-dimensional gel electrophoresis identified five protein families, with a total of nine charged proteins of molecular weights approximating 66, 54, 50, 41 and 34 kilodaltons, that were not present or present in reduced quantities in column fractions that did not inhibit motility. Isolation of individual proteins using preparative gels and HPLC have permitted N-terminus peptide sequencing. Identification of motility inhibitory protein may prove it the first substance discovered that is produced by a more differentiated component of a neoplasm that directly inhibits a metastasis associated property.

Metalloproteinase Gene Structure and Regulation; Matrix Metalloproteinases and Tumor Invasion

B1-100 DIFFERENTIAL INHIBITION OF GELATINASES A AND B BY TIMPs IS REVEALED BY REVERSE ZYMOGRAPHY, Paul P. Beaudry, Kevin J. Leco, Terrina Dickinson-Laing and Dylan R. Edwards, Department of Pharmacology and Therapeutics, University of Calgary Faculty of Medicine, Calgary, Alberta, Canada.

Tissue Inhibitors of Metalloproteinases (TIMPs) are secreted proteins which control the rate of extracellular matrix (ECM) degradation by inhibition of matrix metalloproteinases (MMPs). Disturbance of the MMP/TIMP balance either by overproduction of MMPs or down-regulation of TIMP expression has been associated with invasive and metastatic behavior. Three TIMPs are known: differences in their patterns of expression and biochemical characteristics indicate that they have specific physiological roles. TIMP activity can be visualized using reverse zymography, a form of protease-substrate gel electrophoresis in which gelatin and a source of MMP activity is incorporated into an acrylamide gel. We have carried out reverse zymography using conditioned media that contain predominantly either gelatinase A (72 kDa, MMP-2) or gelatinase B (92kDa, MMP-9). We have observed that while gelatinase A is broadly inhibited by TIMP-1, -2 and -3, gelatinase B is selectively inhibited by TIMP-3. Experiments are underway to verify the significance of this observation *in vivo*. (Supported by the Medical Research Council of Canada, the D.Cameron/J.Barclay-Millar Memorial Endowment, and the Alberta Cancer Board).

B1-101 ANALYSIS OF TISSUE INHIBITOR OF METALLO-PROTEINASES-1 (TIMP-1) TISSUE SELECTIVE 5′ ENHANCER, John J. Caterina and Jeffrey A. Engler, Department of Biochemistry and Molecular Genetics, Schools of Medicine and Dentistry, University of Alabama at Birmingham, Birmingham, AL 35294

In order to identify *cis*-acting elements and *trans*-acting factors responsible for different levels of TIMP-1 production by various cell types, genomic sequences 5′ to the TIMP-1 coding region have been isolated and tested in transient transfection assays using the firefly luciferase system. A 0.5 kb enhancer fragment stimulates TIMP-1 expression >10 fold in cells which produce significant amounts of endogenous TIMP-1, but no stimulation is seen in cells in which endogenous TIMP-1 production is minimal. DNaseI footprinting shows multiple protein/DNA interactions within this enhancer region: some which have been predicted previously as well as others in various unpredicted locales. Our reporter constructs were unaffected by over-expression of the transcription factor XBP-1 or its antisense mRNA product in transient expression assays, suggesting that if XBP-1 is involved in TIMP-1 regulation its area of interaction lies outside of our constructs or XBP-1 is involved at a step of regulation other than transcriptional enhancement. Supported by NIH grants DE08228 and DE10631.

B1-102 MUTATIONAL ANALYSIS OF THE CONSERVED NH_2-TERMINAL DOMAIN OF THE TISSUE INHIBITOR OF METALLO-PROTEINASES (TIMP-1), Nancy C. M. Caterina[‡], L. Jack Windsor[‡*], M. Kirby Bodden[§], Audra E. Yermovsky[‡], Kenneth B. Taylor[‡], Henning Birkedal-Hansen[*], and Jeffrey A. Engler[‡*], Departments of Biochemistry and Molecular Genetics[‡], of Oral Biology[*] and of Restorative Dentistry[§] and the Research Center for Oral Biology, Schools of Medicine and Dentistry University of Alabama at Birmingham Birmingham, AL 35294 Mutants in the tissue inhibitor of metalloproteinases-1 (TIMP-1) protein have been created by site-directed mutagenesis and expressed in HeLa cells, using a recombinant vaccinia virus system. Removal of either or both glycosylation sites yielded proteins which retained wildtype inhibitory activity against both human fibroblast collagenase (FIB-CL) and Mr 72K gelatinase (GL). The "tiny-TIMP" COOH-terminal deletion mutant that lacks the last 57 residues was also inhibitory, but the dose response curves and kinetic analysis suggested that the interaction with the activated enzyme had been altered. A number of replacement mutants in the highly conserved NH_2-terminal domain, including replacement of P5A and P8A or a double mutation in the VIRAK sequence which is absolutely conserved in all TIMPs in all species (VIRAK to VIAAA), also yielded functional proteins capable of inhibiting FIB-CL and Mr 72K GL and of forming SDS-resistant complexes with FIB-CL. Kinetic analysis of inhibition of FIB-CL cleavage of a fluorescent peptide revealed moderate but significant increases in K_I by some but not all of these mutants. Although none of the above manipulations abolished inhibitory function, a functionally inactive mutant was generated by replacement of Cys1 with Ser; the measured K_I for this mutant was 10 fold higher than that for wildtype TIMP-1. Analysis of the structure of the Cys1 mutant by circular dichroism revealed only minor changes, suggesting that the change in K_I was not due to drastic changes in the shape of the molecule. Supported by NIH grants DE08228 and DE10631.

B1-103 RAT NEOPLASTIC EPITHELIAL CELL LINES: EXPRESSION OF GENES ENCODING MATRIX METALLOPROTEINASES AND TISSUE INHIBITORS OF METALLOPROTEINASES, Kathryn L. Gibbons+, Robert L. Raison+, Robert L. O'Grady+ and Anita A. Piper# +Department of Pathology and Immunology and #Department of Biochemistry and Physiology, University of Technology, Sydney, N.S.W. 2065 Australia

Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are produced by both non-neoplastic and neoplastic cell lines. It is believed that in pathological conditions such as tumour invasion and metastasis the ratio of MMPs to TIMPs is elevated. We have a range of rat mammary carcinoma cell lines, BC1, BC4 and BC5 which differ in their invasive and metastatic behaviour *in vivo*. In addition, we also have a non-invasive cell line, A5P/B10 and a malignant cell line, T952/F7 resulting from the transfection of A5P/B10 cells with BC1 genomic DNA.

We have sequenced completely the cDNAs encoding rat TIMP-1 and rat TIMP-2, isolated from a cDNA library prepared from BC1 mRNA. The deduced amino acid sequence for rat TIMP-1 is quite different to human, bovine, murine and rabbit TIMP-1 sequences. Rat TIMP-1 contains three potential asparagine-linked glycosylation sites compared to two in the other species and is longer by an additional ten to twelve amino acids at the C-terminal. The function of these extra amino acids is not yet known but they may be involved in interactions with rat progelatinase B and gelatinase B. In contrast, the rat TIMP-2 deduced amino acid sequence is very similar to human (four amino acid differences) and murine (two amino acid differences) TIMP-2.

In order to investigate the ratio of MMPs to TIMPs the cDNAs encoding rat TIMP-1 and TIMP-2, together with those encoding the MMPs, rat collagenase-3 and rat stromelysin-1 were used to investigate the expression of these genes in both the neoplastic epithelial cell lines of our model system and their derived tumours.

B1-104 MATRIX METALLOPROTEINASES AND SERINE PROTEASES IN HUMAN LEUKEMIC CELLS: RELATION TO CELLULAR INVASION. Anna Janowska-Wieczorek, Haroon R. Hashmi and Greg Sawicki. Dept. of Medicine, University of Alberta, Edmonton, AB, Canada T6G 2R8

Augmented activity of matrix-degrading metalloproteinases (MMPs) and serine proteases is implicated in the invasion of tissue barriers by metastatic tumor cells. With respect to leukemia, we recently reported that myeloblastic KG-1 cells displayed a pronounced capacity to penetrate the reconstituted basement membrane (Matrigel) in the *in vitro* invasion assay whereas erythroleukemic HEL cells were almost noninvasive. In the present investigation, using substrate SDS/PAGE (zymography), we monitored the presence of gelatin- and casein-degrading activities expressed by leukemic cells and determined their specificity. As evaluated in quantitative zymography, KG-1 cells expressed more strongly than HEL cells the membrane-associated gelatinolytic activities of approximate MW 92 and 72 kDa. Moreover, KG-1 cells secreted into conditioned media the 70 and 53 kDa gelatinases and, after treatment with plasmin, an array of gelatin-degrading enzymes (70–30 kDa). In contrast, HEL cells secreted only the 53 kDa gelatinolytic species and after activation with plasmin yielded four minor activities of MW 53 to 30 kDa. The activities of the two membrane-associated, as well as of the secreted 70 and 53 kDa species, expressed by both cell lines were totally suppressed by 1,10-phenanthroline, a specific inhibitor of MMPs, whereas the species of 50, 42 and 40 kDa secreted by KG-1 and HEL cells were fully inhibited by serine protease inhibitors. In addition, we showed that *in vitro* invasion of the reconstituted basement membrane by KG-1 cells was inhibited up to 80% by the inhibitors of metalloproteinases (rhTIMP-2 or 1,10-phenanthroline) and up to 95% by inhibitors of serine proteases (benzamidine or phenylmethylsulfonyl fluoride). Furthermore, kinetic studies of the production and transport of these enzymes proved that only membrane-associated MMPs and secreted serine proteases are essential for penetration of reconstituted basement membrane. In conclusion, our results indicate that the enzymes implicated in tumor metastasis may also be linked to the invasiveness of leukemic cells.

B1-105 **ELEVATED EXPRESSION OF A 160 kDa EXTRACELLULAR MATRIX-DEGRADING PROTEASE (SEPRASE) IN HUMAN BREAST CANCER**, Soheila Korourian, Sophia Kechelava, Jeffery A. Goza, and Thomas Kelly, Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Malignant cells have increased extracellular matrix-degrading protease activity that apparently promotes tumor cell invasion into adjacent tissues. The expression of a 160 kDa membrane-associated protease, termed seprase, in human malignant, benign and normal breast tissues was investigated by immunohistochemistry on formalin-fixed, paraffin-embedded specimens. The polyclonal antibody used in this study was elicited against a pure 100 kDa protein component of chicken embryo seprase and specifically recognizes both active 160 kDa seprase and its proteolytically inactive 100 kDa subunit. Surgical specimens of human breast tissue (43 cases) were stained including: 28 invasive ductal carcinomas, 6 carcinomas in situ, 5 fibrocystic changes, 3 fibroadenomas and 1 normal breast tissue. Neoplastic cells within invasive and in situ components of ductal carcinomas have significantly higher levels of staining than stromal cells and morphologically normal epithelium in the same specimen. Ductal carcinoma cells in lymph node metastases were also strongly positive; however, lymphoid tissue was not labeled. Benign fibrocystic changes and fibroadenomas revealed little or no staining. The normal breast specimen was completely devoid of stain. At the cellular level, staining was predominant throughout the cytoplasm of tumor cells with intense labeling of vesicular structures and nuclear membranes. Staining of membrane bridges between cells suggests that seprase is also on the cell surface where it could degrade extracellular matrix. Detergent extracts of three ductal carcinoma cases revealed 160 kDa protease activity by gelatin zymography as well as strong immunoreactivity with the 100 kDa protein and weaker reactivity with 160 kDa seprase on Western blots. Elevated seprase expression by malignant cells suggests that seprase plays an important role in tumor invasion of normal tissues. Supported by American Cancer Society Institutional Research Grant # 187 (SK) and Arkansas Science & Technology Authority Grant # 94-B-30 (TK).

B1-106 **REGULATION OF PROTEASE EXPRESSION DURING EARLY HEART DEVELOPMENT**

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During heart development, the cells of the endocardial cushions undergo an epithelial-mesenchymal transition and migrate into the surrounding extracelluar matrix. Coincident with this phenotypic change is the expression of the serine protease urokinase by the mesenchymal cells. This protease plays an important role in remodeling of the matrix and promotion of cell migration by regulating cell-matrix interactions. In this study we have begun to examine the factors which bind to the urokinase gene promoter and regulate the expression of this enzyme in the developing heart. Protein(s) present in extracts of heart tissue bind to a region of the urokinase promoter containing an Ets-like consensus sequence (AGGAAA). The amount or activity of this protein changes during development parallelling the increase in urokinase activity. Using a gel-shift assay we have detected a 15-fold increase in the amount of protein binding to the sequence CCAACCGCAGGAAACCTGCCTC from stage 18 hearts compared to stage 13. Specific protein-protein interactions may also occur between this and additional sites within the promoter. These results may lead to a better understanding of the inductive factors present in the heart which facilitate the normal morphogenesis of this organ.

B1-107 REGULATION OF 72-kDa AND 92-kDa GELATINASES BY CALCIUM IONOPHORES. Jouko Lohi[1], and Jorma Keski-Oja[1,2], Departments of [1]Virology and of [2]Dermatology and Venereology, University of Helsinki, FIN-00290 Helsinki, FINLAND.

The family of matrix metalloproteinases comprises at least nine different enzymes with different substrate specifities and divergent regulation. The expression of the interstitial collagenase (MMP-1) and stromelysin-1 (MMP-3) genes is enhanced by an increase in the intracellular calcium levels. This can be achieved by calcium ionophores, like A23187 (calcimycin) and ionomycin, and by thapsigargin, an inhibitor of endomembrane Ca^{2+}-ATPase. We have analyzed the effect of calcium ionophores on the expression of 92-kDa gelatinase (MMP-9) in human fibrosarcoma cells (HT-1080). Ionomycin (10 nM) reduced the levels of 92-kDa gelatinase mRNA in both untreated and PMA or TNFα treated cells, and at 500 nM the mRNA of 92-kDa gelatinase was barely detectable. Decrease in mRNA levels was paralleled by a dose dependent decrease of secreted gelatin binding 92-kDa protein and gelatinase activity as determined by metabolic labeling followed by SDS-PAGE and by gelatin zymography, respectively. Pericellular gelatinolytic activity, as measured by the ability of cultured cells to release radioactivity from culture plates coated with ^{3}H-gelatin, was increased by PMA, and this increase could be prevented by co-treatment with ionomycin. Treatment of the cells with A23187 or thapsigargin caused a similar decrease in 92-kDa gelatinase mRNA and protein levels. Gel retardation analysis of nuclear extracts showed no regulation of the levels of AP-1 and NF-κB binding activities by ionomycin. The expression of 72-kDa gelatinase was only slightly decreased by ionomycin. Treatment of HT-1080 cells with PMA or TNFα resulted in the conversion of 72-kDa gelatinase proenzyme to 64 and 62-kDa forms, presumably active forms of 72-kDa gelatinase. Co-treatment with PMA/TNFα and ionophores resulted in the disappearance of the 64 and 62-kDa gelatinolytic proteins suggesting inhibition of PMA-induced gelatinase activation by ionophores. In contrast, treatment of rabbit synovial fibroblasts with calcium ionophores has been reported to result in endogenous activation of interstitial collagenase. The results suggest negative regulation of metalloproteinases with gelatinolytic potential by calcium ionophores.

B1-108 NOVEL STRATEGIES FOR THE INHIBITION OF GELATINASE A AND B BY PEPTIDES DERIVED FROM THE PROENZYME FRAGMENT.

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The matrix metalloproteinases are a family of enzymes which have evolved to degrade specific components of the extracellular matrix (ECM). This dissolution of the ECM is associated not only with normal tissue remodelling but also in the pathological states of arthritis, peridontal disease and tumour invasion and metastasis. In neoplasia, the gelatinases are of particular interest due to an observed correlation between elevated levels of activated enzyme and the malignant phenotype.

The generation of active gelatinases is believed to involve the dissociation of Cys^{73} from the active site zinc atom, its replacement by water and the concomitant exposure of the active site. The dissociation of Cys^{73} from the zinc atom in the latent enzyme is a result of the proteolytic cleavage of a propeptide domain which contains the conserved amino acid sequence $PRC^{73}GXPDV$ and hence, switches the role of the zinc atom from a non-catalytic to a catalytic one. It has previously been reported that peptides based on this sequence are capable of inhibiting activated gelatinases (Stetler-Stevenson *et al.*, *Amer. J. Med. Sci.* **302**, 163-170, 1991). In our study we have carried out a systematic substitution of Cys by other amino acids known to be effective in chelating the active site zinc atom. These residues include Asp, Glu, His and Pen. Detailed kinetic investigations are currently being carried out and the results will be presented.

B1-109 INVESTIGATION OF COLLAGENASE PRODUCED BY A RAT MAMMARY CARCINOMA CELL LINE, BC1 : REGULATION OF EXPRESSION AMONGST CELL SUB-POPULATIONS.

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The BC1 cell line was derived from a spontaneously arising mammary carcinoma in a Dark Agouti rat. It has been an invaluable tool in the study of the matrix metalloproteinases, in particular collagenase, due to its ability to secrete significant quantities of this enzyme. BC1 is polymorphic, consisting of two subpopulations of cells designated as epithelioid (E) and myoepithelioid (M). The isolation of these cell types through differential trypsinisation and through cloning has allowed for the investigation of interactions between the E and M cells. Co-culture experiments reveal interactions resulting in an increase in collagenase activity in the culture supernatant as well as an increase in the adhesion of M cells to the culture surface. To address whether the higher levels of collagenolytic activity in co-cultures is due to an elevation in the production of this enzyme, collagenase mRNA and protein have been quantitated. An adhesion assay has been developed to investigate the altered M cell adherence. It has been used to determine whether this is due to the direct action of an attachment factor or to increased responsiveness by the M cells. The study of BC1 embraces two areas involved in invasion and metastasis by tumours, specifically, the degradation of connective tissue by the matrix metalloproteinases and altered cell adhesion.

B1-110 REGULATION OF MATRIX METALLOPROTEINASES AND THEIR INHIBITORS IN THE CYTOKINE STIMULATED RAT BRAIN,

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Increased production of 92-kDa type IV collagenase (gelatinase B or MMP-9) has been observed in cerebrospinal fluid samples from individuals with various inflammatory and vascular neurologic diseases. These conditions are associated with increased production of cytokines. We have found that cytokine-stimulated rat brain has increased MMP-9 production and decreased blood-brain barrier permeability. Our present aim is to determine the mechanisms of cytokine regulation of brain metalloproteinases and their inhibitors. Intracerebral injection of lipopolysaccharide (0.125 μg) produced a significantly greater induction of MMP-9 at 8 hrs by zymography, compared to injections of tumor necrosis factor-α (TNF, 5U), interleukin-1β (0.1U), or interferon-gamma(1.5U). Levels of MMP-9 were significantly higher 8 hrs after TNF-α (10^4U) injection, as compared to controls, and remained elevated for 24 hrs. Tissue inhibitor of metalloproteinase-1 was detected in Northern blot assay, 24 hrs after TNF-α injection. Type IV collagen is found in the basal lamina surrounding brain endothelium. Metalloproteinases are involved in the cytokine-mediated proteolysis of the blood-brain barrier. Endogenous as well as synthetic inhibitors of the metalloproteinases may prove beneficial for therapeutic intervention in various brain-related injuries and diseases.

B1-111 GELATINASE A IN THE CYTOSOL OF NORMAL AND TRANSFORMED SHE CELLS ; ITS POSSIBLE INVOLVEMENT IN THE THE POST-TRANSCRIPTIONAL REGULATION OF THE CARCINOGENIC BIOMARKER, ORNITHINE DECARBOXYLASE.

Nguyen-Ba Giao*, Dhalluin Stephane, Ledain Arnaud**, Tapiero Haim*, Elias Zoe°, Poirot Odile° and Hornebeck William°°.**

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A 72 kDa neutral proteinase was identified in the cytosolic fraction of syrian hamster embryo cells (SHE) by gelatin zymography - This intracellular endopeptidase was sensitive to inhibition by metal chelators, but its activity remained unaffected by serine active site agents and N-ethyl-maleimide. It was strongly activated by p-amino-phenylmercuric acetate (APMA) and reacted in Western blots with antibodies raised against human gelatinase A. SHE cells have been transformed by the action of carcinogen Nemalite (10μg/ml). The transformed cell line (SHE.Tr) overexpressed ornithine decarboxylase (EC.4.1.1.17, ODC), the rate limiting enzyme in polyamine biosynthesis closely associated with cell transformation and oncogenesis. In parallel the cytosolic protease level increased twofold as compared to control cells.

In SHE cells staged exposed to some non-genotoxic carcinogens within the framework of the two-stage carcinogenic process, for example Clofibrate (10μg/ml) followed by TPA (0.1μg/ml), both ODC and cytosolic protease were over induced. On the contrary treatment of SHE-Tr cells by α-difluoromethylornithine (0.05mM) dropped ODC to very low level, stimulated however cytosolic protease, the cell proliferation rate decreased. In turnover studies, treatment of cell cultures by Dexamethasone inhibited protease activity and the stability of ODC was increased. In vitro, when cytosolic protein was incubated at 37°C, in the presence of 2mM$CaCl_2$, the ODC activity decreased by 50% after 30 mn incubation. Further decrease was obtained when APMA was added. In contrast the addition of EDTA restored the ODC activity completely.

The results point to a possible involvement of a 72 kDa cytosolic metalloprotease in the post-transcriptional regulation of ODC, consequently in the control of cell proliferation and cell transformation.

B1-112 REGULATION OF 92-kDa GELATINASE RELEASE IN HL-60 LEUKEMIA CELLS: TUMOR NECROSIS FACTOR-α AS AN AUTOCRINE STIMULUS FOR BASAL AND PHORBOL ESTER INDUCED SECRETION, Christian Ries and Petro E. Petrides, Molecular Oncology Laboratory, Dep. of Medicine III, University of Munich Medical School Großhadern and GSF Forschungszentrum für Umwelt und Gesundheit, Munich, Germany

Matrix metalloproteinase 9 (MMP-9), also known as 92-kDa type IV collagenase/gelatinase, is believed to play a critical role in tumor invasion and metastasis because of its ability to degrade type IV collagen, a major structural component of basement membranes. Here we report, that MMP-9 was constitutively released from the human promyelocytic cell line HL-60 as determined by zymographic analysis. Tumor necrosis factor-α (TNF-α) or the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA) enhanced the enzyme release 3-4- or 4-6-fold, respectively. Gelatinase induction by TNF-α and TPA was inhibited by actinomycin D or cycloheximide. Neutralizing monoclonal antibodies to TNF-α (anti-TNF-α) decreased the basal MMP-9 release of these cells. In addition, these antibodies also significantly interfered with the TPA-induced enzyme release. Agents that inhibit TNF-α expression in HL-60 cells such as pentoxifylline and dexamethasone, completely abrogated both the constitutive and TPA-evoked MMP-9 release. Diethyldithiocarbamate (DDTC), which is known to stimulate TNF-α production in HL-60 cells exerted a positive effect on MMP-9 release in untreated cells but was inhibitory in TPA-induced HL-60 cells. The protein kinase C (PK-C) inhibitor staurosporine at low concentrations (100 ng/ml) caused a significant augmentation of MMP-9 release in untreated cultures, that was blocked by the addition of anti-TNF-α. High concentrations (2 μM) of staurosporine completely abolished the extracellular enzyme activity both in untreated and TPA-stimulated cells. These results suggest, that TNF-α is required for basal and PK-C-mediated MMP-9 release in HL-60 leukemia cells. Thus, MMP-9 secretion may be regulated by TNF-α not only in a paracrine but also in an autocrine fashion, possibly potentiating the matrix degradative capacity of immature leukemic cells in the processes of bone marrow egress and evasion into peripheral tissue.

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B1-113 ABNORMAL STEROIDAL REGULATION OF STROMELYSIN EXPRESSION IN NEOPLASTIC HUMAN ENDOMETRIUM. William. H. Rodgers, Sharon Samuel, Victor Okoh and Lynn. M. Matrisian. Departments of Cell Biology (LMM), Vanderbilt University, Nashville, TN 37232; and Pathology, The University of Alabama at Birmingham, Birmingham, AL 35294

Matrix-degrading metalloproteinases (MMPs) are a tightly regulated family of enzymes whose patterns of expression suggest an active role in endometrial structural remodeling and the tissue breakdown associated with menstruation. In the normal endometrium, MMPs are expressed in specific cell types and change with the stage of the menstrual cycle, suggesting regulation by steroid hormones (JCI 94,946). For example, the stromelysins, which are detected in the proliferative and premenstrual/menstrual interval are not detected in the progesterone dominated secretory phase of the menstrual cycle. In vitro studies support steroidal MMP regulation, i.e. progesterone inhibits expression of matrilysin, and stromelysins-1, -2, and -3 in human endometrial explants, and soluble stromal-derived factors mediate progesterone's inhibition of epithelial matrilysin expression in separated stromal-epithelial cultures (PNAS, in press).

Using cRNA *in situ* hybridization we have determined the cellular localization of expression of matrilysin; and stromelysins -1,-2, and -3 in archival diagnostic specimens and explant cultures of neoplastic human endometrium. The patterns and intensity of the MMP hybridization signals in hyperplasia without atypia resembled those of normal proliferative endometrium, while those in atypical hyperplasias and invasive carcinomas were more intense and variable than in normal endometrium. Explants of 4 invasive carcinoma specimens had patterns of MMP expression similar to the surgical specimens. Unlike explants from normal endometrium, two of the carcinoma explants failed to show inhibition of matrilysin and stromelysin RNA expression by progesterone. These results suggest that steroid mediated stromelysin expression in endometrial carcinomas is abnormal and variable, depending on the tumor. Further studies, particularly studies of stromal epithelial interactions associated with steroidal regulation of MMP expression in endometrial tumors, may provide insight into the rolls of MMP expression in invasion of endometrial carcinomas as well as into the biological behavior of individual tumors.

B1-114 A MEW MEMBER OF MATRIX METALLOPROTEINASE EXPRESSED ON THE SURFACE OF INVASIVE TUMOR CELLS WHICH ACTIVATES GELATINASE A Hiroshi Sato[1], Takahisa Takino[1] Yasunori Okada[2] Jian Cao[1], Akira Shinagawa[3] and Motoharu Seiki[1] [1]Department of Molecular Virology and Oncology, [2]Department of Molecular Immunology, Cancer Research Institute, Kanazawa University, Kanazawa 920, [3]Fuji Chemical Industries, Ltd. Takaoka 933, Japan

Gelatinase A (72-kDa type IV collagenase) is believed to be crucial for tumor invasion and metastasis, acting by degrading extracellular matrix macromolecules such as type IV collagen. Gelatinase A is secreted as an inactive precursor (pro-gelatinase A) and activated via the proteolytic cleavage of pro-domain. This activation takes place specifically on the surface of tumor cells and is sensitive to inhibitors of matrix metalloproteinases (MMPs). Thus, the hypothetical activator of pro-gelatinase A is a new member of MMP which bounds to plasma membrane. We have isolated a cDNA which encodes a novel MMP with a potential transmembrane domain (membrane type-MMP, MT-MMP). Expression of MT-MMP induced specific activation of pro-gelatinase A *in vitro* and enhanced cellular invasion of the reconstituted basement membrane Matrigel. Tumor cells of invasive lung carcinomas, which contain activated forms of gelatinase A, were found to express the transcript and the gene product. The new MMP may thus trigger invasion of tumor cells by activating pro-gelatinase A on the tumor cell surface.

B1-115 MMP-1 EXPRESSION IN NORMAL TISSUE AND IN FOS-INDUCED BONE TUMORS, Rüdiger Vallon*, Sabine Gack*, Heather Stanton[$], Jan Tuckerman*, Marina Schorpp*, Jörg Schmidt[§], Gill Murphy[$] and Peter Angel*, * Kernforschungszentrum Karlsruhe, Institut für Genetik, Postfach 3640, 76021 Karlsruhe, Germany, $ Strangeways Research Laboratory, Cambridge CB1 4RN, United Kingdom, § GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Molekulare Virologie Neuherberg, 85758 Oberschleißheim, Germany

Interstitial collagenase (MMP-1) belongs to the Matrixmetalloproteinase family, whose members are products of related genes and share structural and functional properties but differ in substrate specificity. MMP-1 is the only enzyme that is able to efficiently cleave native collagen types I, II and III. We and others have shown that, in tissue culture cells of human origin, MMP-1 expression is stimulated in response to carcinogens, oncogenes and tumor promoters such as TPA. There is a large body of evidence that the AP-1 site of the human MMP-1 promoter, recognised by Fos/Jun dimeric complexes, is the most crucial element for its transcriptional regulation. To address the function and regulation of MMP-1 in a multicellular animal system, we have cloned murine MMP-1 cDNA and genomic sequences. Despite the overall lack of sequence homology, the promoter of the murine MMP-1 gene contains a high affinity AP-1 binding site which reflects evolutionary selection for a common regulatory mechanism. The presence of the AP-1 binding site is likely to be responsible for the strongly enhanced expression of MMP-1 in c-Fos transgenic mice, and, in particular, in Fos-induced bone tumors. In order to identify the cells responsible for MMP-1 expression we raised polyclonal antibodies against a GST-fusion protein in rabbit and sheep. Immunohistochemistry and in situ hybridization analysis revealed that MMP-1 expression is associated with ossification during embryogenesis and in adult mice. The importance of *cis*-acting elements of the murine MMP-1 promoter for regulation of MMP-1 expression in response to cytokines involved in bone homeostasis (TNFα, PTH, Vit D) will be discussed .

B1-116 CATALYTIC DOMAINS OF METALLOPROTEINASES, L. J. Windsor, D. L. Steele, S. B. LeBlanc, K. M. Bodden, H. Birkedal-Hansen, and J. A. Engler, Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham, AL 35294

Metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases which play major roles in extracellular matrix remodeling as it occurs in health and disease. This family includes the collagenases, stromelysins, gelatinases, matrilysin, and macrophage metalloelastase. Structurally, the MMPs consist of a series of distinct and highly conserved domains. These domains include (i) a hydrophobic *signal peptide* that is cleaved off during secretion followed by (ii) a *propeptide* that is essential for maintaining the latency of the enzyme and is apparently removed during activation; (iii) a *catalytic domain* containing the highly conserved zinc-binding site, joined by (iv) a *proline-rich hinge region* to (v) a *hemopexin-like carboxyl-terminal domain* that may play a role in directing substrate specificity.

The catalytic domain, along with the propeptide of fibroblast-type collagenase (mini-CL), stromelysin-1 (mini-SL-1), and matrilysin have been expressed in *E. coli* to determine if the catalytic domains of these enzymes are functionally identical. Mini-CL, mini-SL-1, and matrilysin were expressed as catalytically competent latent enzymes in *E. coli*. They all displayed catalytic activity on a β-casein zymogram and all were activatable with trypsin and organomercurials. However, the mechanisms of activation were slightly different from each other and mirrored the parent enzyme. Mini-CL and mini-SL-1 were purified by immunoaffinity chromatography and matrilysin was purified by size exclusion and S-sepharose chromatography. The purified enzymes displayed slightly different characteristics with regard to (i) formation of SDS-stable complexes with tissue inhibitor of metalloproteinases (TIMP), (ii) formation of complexes with α2-macroglobulin, and (iii) catalytic rate on β-casein. This data suggests that there are functional differences within each enzyme's catalytic domain that are characteristic of that enzyme. This work was supported by NIH grants DE 08228 and DE 10631.

B1-117 CLONING, HETEROLOGOUS EXPRESSION AND CHARACTERIZATION OF A HAMSTER GELATINASE A.

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The 72 kDa gelatinase A is a matrix metalloproteinase (E.C.3.4.24.24) that specifically degrades the type IV collagen, one major structural components of the basement membrane. The enzymatic activities of this enzyme have been found to correlate well in tumor metastatic potentials. The catalytic activity of gelatinase A is tightly regulated since it is made as an inactive pro-enzyme form. The inactivity of the pro-enzyme form is caused by a highly conserved sequence of nine amino acids found at the N-terminus of all the collagenases. This peptide contains a free, reactive cysteine residue that forms a zinc ligand in the active site and thus blocks its activity. Upon activation by the membrane activator, the peptide is cleaved off by the "Velcro" mechanism and gelatinase A becomes permanently activated. We have been interested in the structure/function relationships of gelatinase A and its role in tumor metastasis. Using RT-PCR method with a pair of oligonucleotide primers based on a human and mouse gelatinase A cDNA, the catalytic domain of a hamster gelatinase A cDNA was isolated from a highly metastatic hamster glioma cell lines. Nucleotide sequence analysis reveals high degree of sequence conservation between the hamster clone and those of human and mouse. An active form of gelatinase A was obtained using a bacterial expression system and its structure/function relationships are being addressed using site-directed mutagenesis.

Degradative Enzyme Regulation and Invasion

B1-200 A NEW METALLOPROTEASE EXPRESSED BY C6 GLIOBLASTOMA CELLS PLAYS AN IMPORTANT ROLE IN TUMOR INVASION AND CELL MIGRATION, Verena R. Amberger, Tamara Hensel and Martin E. Schwab, Brain Research Institute, University of Zurich, 8029 Zurich, Switzerland.

CNS myelin and oligodendrocytes have an inhibitory substrate effect on neurite outgrowth and on cell migration of e.g. astrocytes and fibroblasts (Schwab and Caroni, 1988). Glioblastomas, however, infiltrate the whole brain and are found to migrate preferentially on white matter fiber tracts. To investigate this invasive behaviour we choose the C6 glioblastoma cells. Their ability to spread and migrate on CNS myelin is correlated with the expression of a proteolytic activity which is able to inactivate the myelin associated inhibitors. Using various protease blockers the activity was identified as a metalloprotease (Paganetti et al., 1988). We developed a specific peptide degradation assay to characterize the protease (Amberger et al., 1994). The metalloprotease is tightly bound to the plasma membrane and can be solubilized only with detergents, e.g. Chaps or Triton X-100. It is insensitive to blockers of the serin-, aspartyl- and cysteineproteases, but highly sensitive to phosphoramidon. Thiorphan has no effect on the proteolytic activity showing an important difference to endopeptidase 24.11 and endopeptidase 24.18. The C6 metalloprotease is stabilised by dithiothreitol unlike the dithiothreitol-sensitive endothelin converting enzyme (ECE). The purification protocol includes a membrane preparation, a detergent extraction, an affinity column and an anion-exchange column. Testing different human brain tumor cells we found a correlation between the grade of malignancy and the expression of this metalloendoprotease. Additionally glial precursor cells seem to use the same mechanism for migrating in the CNS. Current work is focused on the development of new effective protease blockers with respect to a future medical application.

B1-201 MATRIX METALLOPROTEINASE ACTIVITY IS NECESSARY FOR SMOOTH MUSCLE CELL MIGRATION AFTER ARTERIAL INJURY, M. Bendeck, R. Galardy, M. Reidy, Department of Pathology, University of Washington, Seattle WA 98195, and Glycomed Inc., Alameda, CA 94501.
Smooth muscle cell replication and migration are important for neointimal formation after arterial injury. Matrix metalloproteinases (MMP's) are expressed by many migrating cells. We hypothesized that these enzymes play a role in mediating smooth muscle cell migration through extracellular matrix after balloon catheter injury in the rat carotid artery. Using gelatin zymograms, northern blots, and in situ hybridization we detected induction of a 92kD gelatinase (MMP-9) during the first week after injury, coincident with medial cell replication and migration into the intima. A MMP inhibitor was administered i.p. at a dose of 100 mg/kg/day for 4 days after balloon catheter injury. This caused a significant decrease in the number of SMCs that migrated into the intima, from 99.7 ± 30.8 cells/mm^2 in controls, to 2.84 ± 0.79 cells/mm^2 in MMP inhibitor treated animals. Medial SMC replication rate was not affected by inhibitor. When rats were treated for 10 days after balloon injury, intimal cross-sectional area was decreased from 0.095 ± 0.030 mm^2 in controls, to 0.035 ± 0.018 mm^2 in inhibitor treated rats. By contrast, the number of BrdU-labeled intimal SMCs was not significantly different in the control and inhibitor treated groups. We inferred that the decrease in lesion area was the result of a decrease in the number of SMCs migrating into the intima. We conclude that the expression of MMP's is necessary to facilitate smooth muscle cell migration, and enhances neointimal lesion formation after denuding arterial injury.

B1-202 **DIFFERENTIATION *IN VITRO* OF AN INVASIVE SMALL CELL LUNG CARCINOMA CELL LINE LEADS TO ACQUISITION OF AN ADHERENT, NON-TUMORIGENIC PHENOTYPE.**
Walter T. Dixon* Maryam Varedi** Renate Meuser*Gordon E. Searles*** and Andrew R.E. Shaw#, Department of Agriculture, Food and Nutritional Science*, Department of Surgery**, Department of Medicine*** and Department of Oncology# University of Alberta, Edmonton, Alberta, Canada. T6G 2P5.

NCI H69 small cell lung carcinoma (SCLC) cells grow *in vitro* as multicellular aggregates which are non-adherent to the plastic of the tissue culture flask (H69-C). These cells were highly tumorigenic when injected subcutaneously into severe-combined-immune-deficient (SCID) mice. Following continuous exposure to bromodeoxyuridine (12μm) in culture, a stable sub-population of substrate-adherent cells (H69-B) was isolated which were no longer tumorigenic in the SCID mouse assay. These H69-B cells had a strikingly different repertoire of integrin adhesion molecules when compared to the "parental" line H69-C, as judged by flow cytometry. Northern analysis comparing the two cell types revealed dramatically different expression of mRNA for cytoskeletal and extracellular matrix proteins such as alpha-actinin, fibronectin and collagen, as well as regulators of extracellular matrix remodelling such as collagenase (Type 1), TIMP-1 and TGF- beta. Interestingly, the H69-B cell line also appeared to greatly upregulate the surface expression of CD10, a neutral endopeptidase implicated in the hydrolysis of bombesin-like peptides known to be important in the autocrine growth regulation of small cell lung carcinomas.These cell lines may therefore provide a useful system for investigating growth regulation, neoplastic transformation and metastasis of a neuroendocrine cell type. Using the differential display -polymerase chain reaction technique (DD-PCR) we are currently cloning candidate genes which are differentially expressed in the two cell lines and which may play a role in the loss or acquisition of invasive or motile cell behavior.

B1-203 ROLE OF THE BASEMENT MEMBRANE PROTEIN NIDOGEN IN TUMOR CELL INVASION, Jay W. Fox and Bojan Dragulev, Department of Microbiology, University of Virginia Health Science Center, Charlottesville, VA 22908
Nidogen (entactin) is a basement membrane glycoprotein which has been determined to play a critical role in the supramolecular organization of basement membranes. Nidogen can form ternary complexes with other basement membrane components such as laminin and collagen IV and laminin and heparan sulfate proteoglycan and thus act as "keystone" in the maintenance of the structural integrity of the basement membrane (1-4). Nidogen is rather susceptible to proteolysis by a variety of proteinases with the most readily degraded region being the linker between the G1 and G2 globular domains (5). Proteolytic degradation of nidogen by invasive cells may be one of the critical steps for productive disruption of basement membrane necessary for migration through the matrix. We have continued our investigations on the nature of nidogen proteolysis by a highly invasive metastatic human melanoma cell line WM164 by determining the sites of nidogen proteolysis which occurs when WM164 cells migrate through basement membranes. Furthermore, we are using site-specific mutagenesis of those sites to alter the proteolytic specificity of nidogen and then assessing the ability of WM164 cells to migrate through the matrix containing the mutagenized nidogen. The results of these studies are the basis of this report.

1. Fox, J. W., Mayer, U., Nischt, R., Aumailley, M., Reinhardt, D., Wiedemann, H., Mann, K., Timpl, R., Krieg, T., Engel, J. and Chu, M.-L. (1991) *EMBO J.* **10**:3137.
2. Aumailley, M., Battaglia, C., Mayer, U., Reinhardt, D., Nischt, R., Timpl, R. and Fox, J.W. (1993) *Kidney International* **43**:7.
3. Reinhardt, D., Mann, K., Nischt, R., Fox, J.W., Chu, M.-L., Krieg, T. and Timpl, R. (1993) *J. Biol. Chem.* **268**:10881.
4. Pschl, E., Fox, J.W., Block, D., Mayer, U. and Timpl, R. (1994) *EMBO J.* **13**:
5. Mayer, U., Mann, K., Timpl, R. and Murphy, G. (1993) *Eur. J. Biochem.* **217**:877.

B1-204 TISSUE KALLIKREIN IN HUMAN BREAST CANCER CELLS, Hans Fritz, Andrea Hermann, Peter Buchinger and Joachim Rehbock, 1st Gynecology Clinic and Department of Clinical Chemistry and Clinical Biochemistry at the LMU Munich, D-80337 Munich, Germany.
Tissue kallikrein with a molecular mass of 25 kDa and an isoelectric point of approx. pH = 6 (in twodimensional western blotting) was found in extracts of humen breast carcinomas. Using immunocytochemical methods with kallikrein-specific antibodies the enzyme could be localized in ductal cancer cells. Kallikrein expression seems to be related to the grade of malignancy because only higher differentiated tumors showed immunoreactivity. Kallikrein was also found in healthy breast tissue.
Tissue kallikrein is known to occur in secretory glands like submaxillary and pancreatic gland and we assume therefore that this enzyme is involved in the production and/or secretion of milk in normal human breast tissue. On the other hand, in the case of breast cancer tissue kallikrein may promote tumor growth and/or invasion. Firstly, kinins liberated from ubiquitously occuring kininogens by kallikrein are potent mitogenic and vasodilatory peptides and may strongly increase vascular permeability as well. Secondly, invasion and metastasis formation may be enhanced by the proteolytic action of kallikrein directly because *in vitro* this serine proteinase is a very potent activator of metalloproteinases such as the 92kDa collagenase and gelatinase. These enzymes have been claimed to be involved in penetration of structural proteins by inflammatory and tumor cells.

B1-205 RECOMBINANT HUMAN TUMOR CELL EMMPRIN STIMULATES FIBROBLAST METALLOPROTEINASE PRODUCTION, Huiming Guo, Marion Gordon, Bryan Toole and Chitra Biswas, Department of Anatomy and Cellular Biology, Tufts Medical School, Boston, MA 02111

Tumor cell-derived collagenase stimulatory factor, renamed extracellular matrix metalloproteinase inducer (EMMPRIN), is a ~58 kDa glycoprotein which is located on the outer surface of human tumor cells and which interacts with fibroblasts to stimulate expression of several matrix metalloproteinases in the fibroblasts. We have recently obtained a full length cDNA encoding EMMPRIN (C.Biswas, Y.Zhang, R.DeCastro, H.Guo, T.Nakamura, H.Kataoka, and K.Nabeshima, submitted). Analysis of the deduced amino acid sequence of EMMPRIN has revealed that it is a member of the Ig superfamily and is identical to human basigin and M6 antigen, proteins of previously unknown function. Since recombinant EMMPRIN produced in bacteria was inactive, we transfected EMMPRIN cDNA into CHO cells. Transfection resulted in appearance of EMMPRIN at the surface of the CHO cells and in extensive posttranslational modification of the EMMPRIN protein. The EMMPRIN was extracted from the CHO cells and immunaffinity purified, then tested for its ability to stimulate fibroblast production of metalloproteinases. Enzyme production was assayed by zymography. Significant increases in 72 kDa gelatinase and stromelysin were obtained in cells treated with the recombinant EMMPRIN as compared to controls. These results indicate that the recombinant EMMPRIN from transfected CHO cells has similar functional activity to the extensively glycosylated tumor-derived protein. We propose that EMMPRIN contributes to the regulation of metalloproteinase production during tumor cell invasion and metastasis.

B1-206 **Spectrum Of Extracellular Matrix Degrading Enzymes in T Lymphocytes Controlled By Substrate Anchorage and ß1-Integrins.**

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Human T cells produce and release fibronectin degrading neutral serine proteases with a molecular weight of 50 kD, 70-80 kD (doublet) and 95 kD and have a cell associated 600 kD fibronectin degrading enzyme. The proteases with m.w. 70-80 kD, 95 and 600 kD also degrade laminin and the 600 kD protease degrades gelatin. In addition, serum free conditioned media from human T cell lines contain a 92 kD gelatinase and low amounts of a 72 kD gelatinase which do not degrade fibronectin. Noteworthy, the expression of ECM-degrading proteases is more prominent in lymphocytes than in non-lymphoid tumor cells and fibroblasts. The expression of the 50, 70-80 and 95 kD proteases is downregulated by anchorage of the cells to substrata such as fibronectin or PLL, as compared to cells grown in suspension. The expression of the 600 kD protease is downregulated by crosslinking of $\alpha 4\beta 1$-integrin receptors on T cells. Thus, T cells express secreted and cell associated matrix degrading enzymes controlled by adhesion and spreading as well as direct signalling via ß1-integrins.

B1-207 CONTACT-MEDIATED INDUCTION OF FIBROBLAST MMP-9, [1]B. Himelstein, [1]D. Dilks, [2]E. Bernhard, [3]H. Sato, [3]M. Seiki, and [4]R. Muschel, Depts. of [1]Pediatrics, [2]Radiation Oncology, and [4]Pathology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104 and [3]Cancer Res. Inst., Kanazawa Univ., Kanazawa, Japan.

Matrix metalloproteinases (MMPs) are frequently induced in the stroma of malignant tumors. We are studying the ability of metastatic tumor cells to induce expression of MMP-9 in normal fibroblasts. Nude mouse tumor explants derived from a metastatic transformed rat embryo cell line, 2.8, contained MMP-9. Explants from a non-metastatic transformed rat embryo cell line, RA3, did not. Neither cell line alone in culture expressed MMP-9. In situ hybridization of these tumors revealed MMP-9 mRNA in the stroma of 2.8-derived tumors, but not in RA3-derived tumors. To explore host-tumor cell interactions responsible for MMP-9 expression in this system, co-cultures of these rat sarcoma cells with rat embryo fibroblasts (REF) were performed. Co-culture of 2.8 cells with REF resulted in MMP-9 release into the medium, while co-culture of RA3 cells with REF did not. Further experiments demonstrated that this MMP-9 release was derived from the fibroblasts, appeared to depend upon cell-cell contact with a tumor cell surface protein, and was transcriptionally regulated. Co-culture of 2.8 tumor cells with REF transiently transfected with a plasmid construct linking the 2.2kb MMP-9 promoter to the CAT reporter gene (plasmid 2.2CAT) demonstrated an approximately 5-fold increase in CAT activity when compared to levels in REF alone. As shown below, CAT activity derived from 2.8 cells co-cultured with REF transiently transfected with 5'-deleted MMP-9 CAT reporter constructs decreased by at least 50% when the NF-κB consensus site was transected (599CAT), while no effect was seen on basal expression. CAT activity was abolished in both resting and contact-stimulated REF if only the proximal AP-1 site and TATA consensus (90CAT) or the TATA consensus alone (73CAT) were present. These results suggest divergent transcriptional regulation of MMP-9 expression in resting fibroblasts and in fibroblasts responding to tumor cell contact.

	PERCENT CAT ACTIVITY (relative to plasmid 2.2CAT in REF alone)			
REF alone	100	103	0	0
REF+2.8	513	232	0	0
	2.2CAT	599CAT	90CAT	73CAT

B1-208 LIPOPHILIC GLYCOSAMINOGLYCANS (LIPOGAGS) : INTERACTION WITH SERINE PROTEINASES AND MATRIX CONSTITUENTS. THEIR USEFULNESS FOR CONTROLLING CANCER CELL INVASION. **Hornebeck W., Caruelle J.P., Barritault D. and Meddahi A., CRRET Laboratory, URA CNRS 1813, University Paris XII, Créteil, France.**

A set of neutral proteinases acting in a cascade fashion is involved in the invasion of host tissue by tumor cells. We design compounds which exhibit a dual property : i) they can inhibit several serine proteinases involved in matrix metalloproteinases activation ii) They can bind to matrix constituents and confer them protection against proteolysis.
We previously showed that heparin was a fast acting tight binding hyperbolic, non competitive inhibitor of neutrophil elastase (Ki =75 pM); it also inhibited Cathepsin G but less efficiently (1,2). We first synthesized several fatty acyl glycosaminoglycan derivatives. A member of this series N-Oleoyl (1,3)-heparin inhibits neutrophil elastase as efficiently as heparin (Ki = 90 pM), it is also a powerful inhibitor of Cathepsin G (Ki < 10 pM), and on contrary to heparin, it inhibits urokinase and plasmin with Ki in nanomolar range (3). The presence of a fatty acid moiety in this molecule allowed it to bind to elastin (Kd = 10^{-6} M) and LipoGAG-elastin complexes were refractory to hydrolysis by elastolytic enzymes (4). We further synthesized CarboxyMethyl Dextran BenzoSulfonates (CMDBS) of different molecular weight and degrees of substitution in benzosulfonate groups. CMDBS with high BS substitution inhibited neutrophil elastase (K_i < 10 pM) and plasmin (IC50 = 10.5 µg/ml). They also interacted with human fibronectin and FGF2 further protecting these matrix constituents against proteolysis (elastase, plasmin, trypsin...) (4). LipoGAGs possess the ability to inhibit uPA, plasmin, leucocyte proteinases considered as the main initial actors in the proteolytic cascade leading to tumor cell invasion. Their potential to interact with matrix constituents possessing hydrophobic (elastin, collagens) and heparin (fibronectin, laminin, FGFs...) binding sites confers them protective function against proteolysis which could be of therapeutic value in cancer.

1- Redini F et al, Biochem. J. 1988, 252, 515-519.
2- Baici et al, Biochem. Pharmacol., 1993, 46, 9, 1545-1549.
3- Moczar and Hornebeck, Int. J. Biol. Macromol., 1991, 13, 261-262.
4- Meddahi et al, Biochem. J. submitted.

B1-209 THE RGD AND CS-1 CONTAINING CELL BINDING REGIONS OF FIBRONECTIN SIGNAL OPPOSING EFFECTS ON METALLOPROTEINASE EXPRESSION VIA $\alpha5\beta1$ AND $\alpha4\beta1$ INTEGRINS, Pirkko Huhtala, Martin J. Humphries*, James B. McCarthy**, Zena Werb*** and Caroline H. Damsky, Departments of Stomatology and ***Radiobiology, University of California, San Francisco, CA 94143, *University of Manchester, Manchester, United Kingdom,**University of Minnesota, Minneapolis.

Rabbit synovial fibroblasts (RSF) plated on the RGD containing central cell binding domain of fibronectin (Fn 120) express increased levels of metalloproteinases (MMP) compared to RSF plated on intact Fn. This inductive signal has been shown to be transduced by the $\alpha5\beta1$ integrin (Werb et al. J. Cell. Biol. 109: 877). The goal of the present study was to determine which domains of Fn outside the central cell binding region participate in the regulation of MMP in RSF. To identify these additional regions we exposed RSF to substrates to which specific domains of Fn had been bound along with Fn 120, to determine which combinations of fragments could suppress the induction of MMP promoted by the interaction of $\alpha5\beta1$with Fn 120, and would result in expression of the low levels of MMP found in cells plated on intact Fn. The C-terminal 33/66 K fragment of Fn, which contains both the high affinity heparin binding domain and the IIICS domain, was able to suppress collagenase (MMP-1), stromelysin (MMP-3) and 92 K gelatinase (MMP-9) expression when combined with Fn 120. Synthetic peptides from this region were tested further for their ability to suppress MMP expression. Only the CS-1 peptide in the IIICS region of Fn was able to suppress MMP expression when combined with Fn 120. The CS-1 sequence is a known binding site for $\alpha4\beta1$ integrin and using antibodies against the $\alpha4$ subunit we show that RSF have functional $\alpha4\beta1$ on their surface, and interference with binding of $\alpha4\beta1$ to CS-1 with anti-$\alpha4$ antibodies is able to block the suppressive signal seen with mixed matrix of Fn 120 and CS-1 peptide. We conclude that signaling through $\alpha4\beta1$ has an important role in suppression of MMP expression in RSF. We hypothesize that altered signaling through $\alpha4\beta1$ integrin could account for the constitutively elevated levels of MMP found in transformed cells, even when plated on intact Fn.

B1-210 HUMAN COLON CARCINOMA CELLS SECRETE A POTENT INHIBITOR OF NEUTROPHIL ELASTASE,

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The proteolytic enzyme, cathepsin B, has been implicated in the malignant progression of various human and mouse tumors. In non-pathological situations, this cysteine protease is generally found inside cells, i. e. within the endosomal/lysosomal compartment. It has a broad activity spectrum degrading most proteins in a pH range from 4.0 to 8.0. During progression of malignant tumors cathepsin B has been found to be secreted either as active enzyme or as proenzyme. Cathepsin B has recently been proposed as prognostic marker for breast, colon and lung cancers.

We have shown that tumor cell-secreted procathepsin B can be activated by purified neutrophil elastase (*Biochim. Biophys. Acta* 1226: 117-125, 1994). In this study we addressed two questions: 1. Is neutrophil elastase present in human colon carcinomas, and 2. Does the co-culture of human colon carcinoma cells with neutrophils generate a cathepsin B-dependent pericellular proteolysis as assessed with radiolabeled laminin ? We show that neutrophil elastase is present in colon carcinoma tissue and that its level is in good agreement with the histological degree of infiltration by neutrophils. In co-culture experiments, elastase was found to be released by neutrophils in a cell number-dependent way, but no activation of tumor cell-secreted procathepsin B could be observed. Colon carcinoma cells were found to secrete a physiological inhibitor of neutrophil elastase, α_1PI. The importance of this finding in the context of the pericellular activation of tumor cell-secreted procathepsin B by neutrophil elastase is discussed.

B1-211 STUDIES IN HUMAN BLADDER CANCER PROTEASE ACTIVITY USING A NOVEL ESTERASE ASSAY,

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Protease-mediated degradation of the basement membrane is a critical event in determining the outcome of human bladder cancer, since 30% of patients with T1 tumours show progression to a higher stage, compared with 4% with non-invasive tumours. A novel assay has been developed for the study of serine and cysteine protease activity, based upon a family of synthesised ester substrates, which vary from each other in a single amino acid substitution (Anal Biochem 220: 238-243, 1994). Protease activity is being studied in a series of human bladder cancer cell lines. UCRU-BL-17/2 (BL-17/2) is a heterogeneous human bladder cancer cell line derived from a biopsy of a transitional cell carcinoma, Grade III Stage T4b, following two passages in nude mice. Nine clones established by limit dilution from BL-17/2 show great variation in their biological properties, including in vitro growth rates and morphology, ploidy, and in particular, in vivo tumorgenicity. Three of the clones are non-tumorigenic in nude mice, three are tumorigenic, and three are tumorigenic and invasive. Preliminary analysis of two invasive clones, B8 and B10, indicates that both cell lines have high leucine- and methionine-specific activity within their membrane fractions, with mainly lysine-specific activity within their cytoplasm. Studies are underway to screen the entire panel of clones to compare protease activity across the range of biological activities, and to determine the role of proteases in basement membrane degradation both in vitro and in vivo.

B1-212 SYSTEMATIC MUTATIONAL ANALYSIS OF THE RECEPTOR BINDING REGION OF THE HUMAN UROKINASE-TYPE PLASMINOGEN ACTIVATOR

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The invasive and metastatic capacity of tumor cells depend in part on uPA and uPAR expression. uPA converts plasminogen to plasmin which plays a central role in extracellular proteolysis. Through binding of uPA to tumor cell-associated uPAR the proteolytic activity is focused to the surface of tumor cells and will thereby promote the dissolution of the tumor stroma and the basement membrane. The N-terminal located EGF-like domain of uPA (GFD, aa 1-45) mediates the binding to uPAR: aa 20-30 confer uPAR binding specificity, aa 13-19 may be necessary to attain proper conformation of GFD. We were interested to define essential amino acids within the region between aa 20-30 for the binding of uPA to uPAR.

The amino-terminal fragment of human uPA (ATF; aa 1-135), which contains the binding site to the uPA receptor (uPAR) but lacks its proteolytic domain, was expressed in the yeast *Saccharomyces cerevisiae*. Recombinant yeast ATF, which was modified by an N-terminal in-frame insertion of a His_6 tract, was purified by nickel chelate affinity chromatography and shown to be functionally active since it efficiently competes with uPA for binding to uPAR as determined by flow cytofluorometry. We systematically have mutated the loop between Cys-19 and Cys-31 by substitution of each of the 11 amino acids in the loop with alanine individually or together with other residues. In addition, those residues which retained almost wild-type activity after substitution by alanine were deleted. The mutant proteins were shown to be expressed in a stable form in yeast. Using two different microtiter-plate based assays, we were able to group the different mutant proteins into three classes: i) mutant proteins with no or very little uPAR binding avtivity; ii) mutants with a significant decrease of binding to uPAR and iii) ATF variants with (almost) wild-type uPAR binding activity. These analyses may help to develop uPA-derived peptide analogues as potential therapeutic agents to block the tumor-cell associated uPA/uPAR interaction and therefore, in consequence, tumor invasion and metastasis.

B1-213 THE APPLICATION OF NOVEL SYNTHETIC PROTEASE INHIBITORS FOR THE DISCLOSURE AND PARTIAL CHARACTERISATION OF A CATHEPSIN L/S LIKE PROTEASE PRODUCED BY OSPR OSTEOSARCOMA CELLS IN CULTURE; AND THEIR UTILISATION IN THE PREVENTION OF INVASION.

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Osteosarcoma affects one thousand new patients in the USA each year, most in their late teens or early twenties. If untreated, the primary tumour will always metastasize resulting in a poor prognosis. Since cysteine proteases have been implicated in the invasive process, it was decided to investigated their role in this disease process.

Utilising the biotinylated peptide based affinity label Biotin-Phe-Cys(Benzy)-Diazoketone in conjunction with SDS-PAGE and Western blotting techinques a protease with molecular weight of 25KD was disclosed in the media conditioned by the PROS cell line. This displayed properties similar to the cysteine proteases cathepsin L and cathepsin S. Utilising Boyden chamber invasion assays, the novel inhibitor Z-Phe-Tyr(OButyl)-Glyoxal-a potent inhibitor of cathepsin L, was investigated for its ability to block the invasion of PROS cells through re-constituted basement membranes (Matrigel inserts). It was found that cell invasion was inhibited by the glyoxal at an IC_{50} of 5ηM. The inhibitor was non-toxic to the cells, and also had no effect on the migration of the MG63 osteosarcoma cell-line that did not express the protease.
This represents the first use of this type of peptide based inhibitor in an *in vitro* system.
Further investigation to fully characterize the protease is underway.

B1-214 EXPRESSION IN COS CELLS OF A cDNA ENCODING A NOVEL HUMAN TUMOR FORM OF CATHEPSIN B,

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Cathepsin B is a lysosomal cysteine proteinase that has been implicated in tumor progression and metastasis. Cathepsin B expression is increased and its intracellular trafficking is altered in cancers from humans and rodents. Our laboratory has previously found that cathepsin B pre-mRNA undergoes alternative splicing in some human tumors to produce a message that lacks the sequences for the amino-terminal signal peptide and part of the activation propeptide, implying that its product should not be targeted to the lysosome. Remarkably, this message can be translated *in vitro* and the resulting protein is enzymatically active. We have also shown that this message can be translated *in vivo* by constructing a chimeric cDNA encoding the connecting peptide of proinsulin fused to the C-terminus of the tumor form of cathepsin B. When driven by the SV40 promoter, this cDNA generated high levels of the amino-terminal truncated cathepsin B in COS cells, as detected in western blots using an antiserum to the insulin c-peptide. Since this message has thus far been found only in tumors, and we have now shown that it can be translated into an active protein, we speculate that it may play a role in tumor progression or in the maintenance of the transformed state. Alternatively, it may prove to be an interesting marker of malignant transformation. Work is in progress to explore possible functions of this novel tumor associated isoform of cathepsin B and the relationship between its expression and tumor progression.

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B1-215 THE RETINOBLASTOMA GENE REPRESSES INVASIVENESS AND TUMORIGENICITY AND INDUCES MELANOGENESIS IN MURINE MELANOMA CELLS.

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The retinoblastoma gene is frequently deleted or mutated in many tumor types and all cases of retinoblastoma. Apart from its role in regulation of the cell cycle, the Rb gene product appears to be involved in control of differentiation. Malignant metastatic cells show many properties of poorly differentiated cells, including a the invasive phenotype. We have transfected an Rb cDNA in an expression vector under the control of the β-actin promoter into B16F10 melanoma cells. The cells expressing Rb mRNA, as assessed by RT-PCR, showed reduced growth rates and increased melanogenesis in vitro. The Rb expressing cells also have a reduced capacity to invade through an artificial basement membrane, a key characteristic of metastatic cells. Vector control transfectants showed no alteration of invasiveness. When injected into nude mice, the Rb expressing cells show reduced tumorigenicity and reduced metastatic potential. These data indicate that Rb expression stimulates melanoma cell differentiation and, either directly or indirectly, reduces the invasive potential of these cells.

B1-216 CD9: A MODULATOR OF BOTH B CELL ADHESION AND MOTILITY

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Successful development of B lymphocytes requires mechanisms to arrest precursors within the bone marrow, but allow cells to enter circulation following immunoglobulin gene rearrangement. Leukemia cells leave the marrow prematurely possibly through subversion of these mechanisms. Proteins which are expressed on cells of the B lineage in a maturation-dependent manner are candidates for molecules which may control adhesive, and motile functions governing B cell development. CD9 is a 22 kDa glycoprotein belonging to a unique family of multiply-inserted transmembrane proteins. CD9 is present on normal pre-B cells, lost on circulating B cells, but retained on >90% of childhood acute lymphoblastic leukemias. To investigate the role of CD9 in the adhesion, and motility of B lineage cells we stably transfected a 1.2 kb cDNA encoding CD9 into the CD9-ve B cell line Raji. Cell spreading on extracellular matrices involves extensive cytoskeletal rearrangement which may antagonize locomotion, and prime cells for the receipt of signals governing differentiation and proliferation. The pre-B cell lines HOON, and NALM-6 spread rapidly on a fibronectin matrix whereas the B cell Raji, and the CD9 transfectant did not. In an adhesion assay 15 (+/- 0.15) % of HOON cells bound to fibronectin in comparison to 5 (+/- 1.7) % of Raji, and 5 (+/- 1.8) % of Raji-CD9 transfectants indicating that expression of CD9 *per-se* did not confer fibronectin-binding ability. Exposure to immobilised anti-CD9 mAb promoted extensive spreading of HOON, and NALM-6 cells, but not of Raji. However, Raji-CD9 transfectants spread rapidly on the immobilised antibody indicating that CD9 directs a spreading signal on both pre-B and B cell backgrounds. Random cell motility was compared in Raji and the Raji-CD9 transfectant using a modified Boyden chamber. The CD9-transfectant was found to be 4.43 x as motile as Raji (n=4), and the motility could be reduced 91% by pre-incubation of the cells with an anti-CD9 mAb (70 mg/ml). We conclude that CD9 may play a role in cellular decisions regarding cell motility, and arrest. CD9 may therefore be a target for strategies aimed at containing leukemic spread.

B1-217 **Stromal expression of c-Ets1 transcription factor correlates with tumor invasion**

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The stroma reaction has an important role in tumor growth, invasion and metastasis. In various invasive human carcinomas, as well as in a mouse model for tumor invasion, transcripts encoding the transcription factor c-Ets1 were detected within stromal fibroblasts whereas they were absent in epithelial tumor cells. This expression of c-ets1 was often increased in fibroblasts directly adjacent to neoplasic cells. Endothelial cells of stromal capillaries were also positive for c-ets1 expression. In contrast, fibroblasts of corresponding non-invasive lesions and of normal tissues were consistently negative.
The features of c-ets1 expression in human carcinomas are reminiscent of the pattern observed in chicken and mouse embryos, where c-ets1 transcripts accumulate in the mesenchyme surrounding growing and invading epithelial buds.

Conditioned media of various tumor-derived epithelial cell lines induced c-ets1 expression in cultured human fibroblasts. In these cells, c-ets1 was an early response gene for TNFα and IL1α. The expression of c-ets1 induced by various cytokines in cultured fibroblasts correlated with the amounts of transcripts for potential target genes, such as collagenase-1 and stromelysin-1. The same correlation was observed in some of the invasive carcinomas investigated. Endothelial cells were positive for c-ets1 mRNAs, but in contrast negative for these proteases.

These results suggest that c-Ets1 participates in the regulation of tumor invasion *in vivo* ; it is tempting to speculate that the c-Ets1 transcription factors take part in the transcriptional control of several matrix-degrading proteases gene expression, either independently or in cooperation with other transcription factors.

B1-218 ELEVATION OF HYALURONAN LEVELS AT SITES OF OVARIAN TUMOR CELL ATTACHMENT AND INVASION IN VIVO,

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Ovarian tumor cells frequently metastasize to the peritoneal cavity where they induce effusions in which the tumor cells grow in suspension. Some of the tumor cells attach to and invade into the mesentery and peritoneal wall. Hyaluronan (HA) is a ubiquitous component of extracellular matrices in which cellular interactions, movement, and proliferation take place. We have used the mouse ovarian ascites tumor, MOT, as a model to follow changes in hyaluronan levels during growth and invasion. Subsequent to introduction of MOT cells into the peritoneum, the amount of HA in the ascites rose from an undetectable level to a peak value of ~200mg/l at 9 days. The level of HA then fell rapidly to control levels by day 16. Control thioglycolate ascites contained only small amounts of HA (~1mg/l), suggesting that the highly elevated level of HA in the tumor ascites is tumor-specific. Examination of the mesentery and peritoneal wall showed attachment of small clumps of tumor cells by 3-5 days post-injection, after which large numbers of cells invaded throughout the tissues. Using biotinylated core protein of aggrecan as a specific probe for visualizing HA, we observed local accumulation of HA at the initial sites of attachment of tumor cell clumps, followed by high levels of accumulation throughout the tissues as tumor cell invasion progressed. We conclude that HA accumulation occurs as a result of tumor cell attachment and invasion, presumably due to stimulation of mesothelial or fibroblast HA production, and propose that the HA-rich matrix promotes further tumor cell invasion.

Models of Tumor Adhesion, Invasion and Angiogenesis; Cell Motility Mechanisms

B1-300 STRUCTURAL FEATURES AND COMPLEMENTARY EXPRESSION PATTERN OF TWO DISCOIDIN I RECEPTOR TYROSINE KINASES SUGGEST INVOLVEMENT IN TUMOR INVASION,

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The receptor tyrosine kinases MCK-10 and CCK-2 exhibit a high degree of overall homology and represent members of a novel subclass characterized by a discoidin I motif in the extracellular domain and a long cytoplasmatic juxtamembrane region (JM). Two isoforms of MCK-10 were isolated. They differ by an in frame insertion of 37 amino acids in the JM region, that contain consensus sequences for internalization and SH3 domain interaction and affect receptor kinase activity and extracellular glycosylation. MCK-10 is cleaved within the extracellular domain to yield one fragment containing the kinase domain and one soluble ectodomain including the adhesive motif. Comparative in situ hybridization studies on various tumor sections reveal that both receptors are expressed mutually exclusive in different cell types. Whereas MCK-10 expression is restricted to neoplastic cells, CCK-2 mRNA is predominantly expressed in stromal cells surrounding the invading tumor. The distinct expression pattern and the presence of the discoidin I motif in their extracellular domains suggest that both receptors play an important role in processes involved in cell-cell interactions and possibly tumor invasion and metastasis. The remarkable complexity of MCK-10 isoforms in conjunction with the cleavage of the extracellular domain with putative adhesion properties further supports this hypothesis.

B1-301 BIPHASIC EFFECT (STIMULATION AND SUPPRESSION) BY TENASCIN ON HUMAN GLIOMA CELL MIGRATION.

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Tenascin is an extracellular matrix protein which is expressed in human gliomas. Cell receptors for tenascin are reported to utilize the αv subunit of integrin as one chain of the heterodimer receptor. We tested whether purified tenascin, passively deposited on monolayer surfaces, influenced the adhesion or migration behavior of human glioma-derived cells, SF767. Studies of other ECM proteins (laminin, collagen, fibronectin, vitronectin) demonstrated that adhesion increases in a dose-dependent manner, with optimal (maximum) specific attachment by 30-60 minutes at 37°C using 100 μg/ml. In contrast, glioma adhesion to tenascin increased to a maximum degree at 10 μg/ml, but steadily decreased using coating concentrations of 33 and 100 μg/ml. Cell adhesion to tenascin could be completely blocked (to basal levels) using anti-ß1 antibodies. Surprisingly, treatment with anti-αv antibodies led to slightly enhanced cell adhesion. Using a microliter scale migration assay (Berens *et al*, Clin Exp Mets, 1994) it was found that migration of glioma cells on tenascin was dose-dependently stimulated at coating concentrations of 1 and 3 μg/ml but cell migration was actually suppressed (to rates below that seen on BSA) when tested on 30 or 100 μg/ml. Migration on optimal concentrations of tenascin could be reversibly inhibited by treatment with anti-ß1 antibodies; treatment with anti-αv antibodies actually stimulated glioma migration. We conclude that glioma cells express two separate receptors for tenascin; and that ligand density, determined by different coating concentrations of tenascin, activates these different integrins. The ß1-containing integrin(s) mediate adhesive and migratory responses, while the αv-containing integrin(s) appear to be counteradhesive and inhibitory to migration. These findings highlight the interplay between different integrins which recognize the same ECM protein, and demonstrate that the net response of a cell to complex extracellular matrix ligands is an integrated manifestation of differing, and possibly opposing, integrin-mediated reactions.

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B1-302 THE CELL BIOLOGY OF 5T4, AN ONCOFOETAL ANTIGEN LOCATED AT MICROVILLUS PROJECTIONS OF THE PLASMA MEMBRANE,

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5T4 is a cell surface antigen, defined by a monoclonal antibody (Mab) 5T4, with a tissue distribution limited to foetal trophoblast membranes, low levels in some specialised epithelia, but high expression in many human carcinomas. The presence of 5T4 on colorectal carcinoma cell membranes correlates with increased metastatic spread and poor prognosis. Such an expression pattern suggests a role for 5T4 in invasive processes. The aim of the present study was to investigate the cell biological role of 5T4.

The mouse L cell line was stably transfected with 5T4 cDNA inserted into $pCMV\alpha_{neo}$, or $PCMV\alpha_{neo}$ alone, producing 5T4-positive and -negative clones.

The pattern of distribution of 5T4 on the plasma membrane of the 5T4-positive L cells was determined, by labelling with (Mab) 5T4. By confocal immunofluorescence microscopy, a 'polkadot' pattern of staining was observed, heterogeneous in intensity between cells, but evenly distributed over the entire cell surface. Transmission and scanning electron microscope studies ascertained that 5T4 is concentrated at microvillus projections of the plasma membrane, with little expression in between. Such projections have been associated with cellular interactions. Interestingly, an identical pattern of staining was observed with the EJ colon carcinoma cell line, which naturally expresses 5T4.

Time-lapse video microscopy and various cell biological assays were then employed. The presence of 5T4 on L cells led to a fibroblast-like morphology (compared to epithelial-like), an altered pattern of division, decreased cell/substratum adhesion and increased cellular motility.

The present results support our hypothesis that 5T4 has an active function in the development of tumorigenicity and/or metastasis.

B1-303 TRANSFORMING GROWTH FACTOR-BETA PROMOTES EPIDERMAL GROWTH FACTOR INDUCED CELL MIGRATION AND FOLLICLE NEOFORMATION IN COLLAGEN GEL YET INHIBITS CELL PROLIFERATION,

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Regulation of growth and migration of thyroid follicular cells by epidermal growth factor (EGF) and transforming growth factor beta (TGF-ß1) was studied in primary cultures of porcine thyroid follicles embedded in collagen gel. Cultures were exposed to growth factors and [^{3}H]thymidine (1 μCi/ml) for 3 d and then examined by light microscopic autoradiography. EGF at 1 ng/ml increased the [^{3}H]thymidine labeling index from <1% (control value) to 60% and stimulated the number of cells invading the collagen matrix 5-fold. Intermediate responses were seen after treatment with 0.1 ng/ml of EGF. EGF-stimulated [^{3}H]thymidine incorporation was reduced by TGF-ß1 at concentrations above 0.1 ng/ml. In contrast, TGF-ß1 which alone only had minor stimulatory effects on cell motility, markedly promoted the migratory response to EGF at 1 ng/ml. Thus, addition of 0.1 ng/ml of TGF-ß1 caused a severalfold increase in number of migrating cells without associated effects on [^{3}H]thymidine incorporation. EGF together with 1 ng/ml TGF-ß1 still stimulated cell migration 4-fold over control values despite a strongly inhibited [^{3}H]thymidine incorporation. When EGF and TGF-ß1 were combined, the epithelium of mother follicles became discontinuous in regions of intense cell migration; the number of microfollicles located peripherally to the mother follicles was increased synergistically.

In conclusion, TGF-ß1 modulates the response of porcine thyrocytes to EGF in collagen gel cultures by promoting cell migration but having no (low TGF-ß1) or an inhibitory (high TGF-ß1) effect on [3H]thymidine incorporation. The findings suggest that EGF stimulates cell motility independent of the mitogenic signal. The persistent loss of epithelial integrity during enhanced cell migration indicates that mechanisms of intercellular adhesiveness are down-regulated by TGF-ß1 and EGF in combination.

B1-304 THE PROTOONCOGENE/TRANSLATION FACTOR EIF4E: A SURVEY OF ITS EXPRESSION IN BREAST CARCINOMAS,

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The eukaryotic translation initiation factor eIF-4E binds to the cap structure of mRNAs as one component of the eIF-4 translation initiation complex, which mediates the recruitment of mRNA to the ribosomes. Overexpression of eIF-4E can result in oncogenic transformation and uncontrolled growth of mammalian cells, presumably by facilitating the expression of growth control gene products which are normally translationally repressed. Whereas the mechanism of eIF-4E-mediated transformation is being actively pursued, clinical investigations into the expression of eIF-4E in prevalent human cancers are lacking. We have recently initiated a screen of breast carcinomas by probing with eIF-4E antiserum. Using Western blots, we have analysed the level of eIF-4E in 38 carcinomas, 7 normal samples, and 3 fibroadenomas. We found that eIF-4E was elevated 3 to 10-fold in virtually all the carcinomas we analyzed, but not in fibroadenomas. This analysis was also confirmed by immuno-histological staining *in situ*, showing that overexpression of eIF-4E can be readily identified at the single-cell level. Our results suggest that an elevation of eIF-4E may be an essential component in the development of breast cancer and metastasis.

B1-305 INVASION AND MIGRATION OF COORDINATED CELL CLUSTERS IN RHABDOMYOSARCOMA AND OTHER HEAD AND NECK TUMORS,

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Invasion and metastasis is mainly considered to be the consequence of single cells developing an invasive and migrating genotype. We investigated the cell invasion and locomotion occurring in various primary head and neck tumors during long-term cultivation within three-dimensional (3-D) type I collagen lattices. Based on time-lapse videorecordings, subsequent cell tracking, and data analysis from a primary rhabdomyosarcoma lesion, we provide direct evidence that groups of clustered tumor cells are able to detach from the primary lesion and invade the surrounding tissue. These cell clusters comprising up to 500 and more cells detach from the tumor in a highly coordinated manner and locomote within the 3-D collagen matrix as a coherent group. Whereas single locomoting tumor cells showed little directional persistence, the cell clusters migrated straight forward at speeds between 20-60 μm/h, exhibiting one or several prominent cells constantly forming the leading edge. The onset of invasion was observed within 3-10 days of cultivation in 3-D collagen lattices, wheras no corresponding behavior was obtained using conventional liquid culture systems. The frequency of locomoting cell clusters within a panel of different head and neck tumors (n=40) was 20-30%, most prominently in squamous cell carcinomas and frequently in association with established metastatic disease. In conclusion, the detachment of coordinated cell clusters from primary tumor lesions and subsequent directional migration may be a relatively common pathogenetic feature of invasion and metastasis.

B1-306 INVOLVEMENT OF INTEGRINS IN THE ADHESION OF BREAST CANCER CELL LINES TO BONE MATRIX PROTEINS, Wojciech J. Grzesik[1], Gabri van der Pluijm[2], Pamela Gehron Robey[1], [1]Bone Research Branch, National Institute of Dental Research, NIH, Bethesda, MD 20892, USA and [2]Department of Endocrinology, University Hospital, 2333 AA Leiden, The Netherlands.

Breast cancer frequently metastasizes to bone but the underlying mechanisms for such a tendency remain unclear. In this study we attempted to characterize the patterns of cell attachment of various breast cancer cell lines to potential ligands present in the bone matrix. Furthermore, we have characterized the integrin receptors present on cancer cells. We have also investigated the involvement of particular integrin receptors in adhesion by applying short, RGD-containing peptides as competitive blockers and using specific anti-integrin antibodies for inhibition of cell attachment.
All examined breast cancer cell lines express a broad spectrum of integrin subunits (α_1-α_6, α_v, β_1, β_3), but both qualitative and quantitative differences between various cell lines exist. The highly metastatic (and simultaneously osteotropic) cell line MDA-MB-231 expresses high levels of β_1 integrin compared to less metastatic cell lines. Similarly, all cell lines exhibit high affinity to the bone matrix proteins; however, a distinct and unique pattern of attachment can be attributed to each one of them, reflecting to a certain degree the cell's repertoire of integrins. The attachment of breast cancer cells is predominantly integrin-mediated, as it can be inhibited by short, RGD-containing peptides in solution and specific anti-integrin antibodies. This data strongly support the hypothetical involvement of integrin receptors in the process of metastasis formation in bone during the course of the breast cancer disease. It may also indicate a possible approach towards developing drugs that interfere with the metastatic process.

B1-307 AN ORTHOTOPIC INVASION AND METASTASIS MODEL IN HUMAN/MOUSE CHIMERIC ANIMALS, Meenhard Herlyn, Peter Soballe and Kapaettu Satyamoorthy, The Wistar Institute, Philadelphia, PA 19104

Growth and invasion of malignant cells is increased in an orthotopic microenvironment. However, current human tumor in nude mice models do not account for species-specific interactions between normal and malignant cells. We have developed a new model for invasion and metastasis model in which human melanoma cells are injected into human skin previously grafted to immunodeficient SCID mice. All of seven melanoma cell lines that were tumorigenic after subcutaneous inoculation in mice also proliferated in human skin grafts. Whereas few differences in cell type, nuclear-to-cytoplasmic ratio, or degree of pleomorphism were found between melanomas injected into human skin grafts or subcutaneously in murine skin, major differences were found in their growth patterns. Melanomas WM164, WM9, and 451Lu grew in human skin as multiple nodules and infiltrated the grafts without major architectural changes of the dermis. In contrast, in murine skin these cell lines grew as expanding masses without invasion of the murine dermis. Other melanomas, such as WM852, WM793, or 1205Lu, showed infiltration of the human dermis along collagen fibers with prominent endothelial vessels. Two distinct vascularization patterns of melanomas in grafted human skin were noted. In tumors with a more infiltrative pattern, such as WM852, longitudinal arrays of intratumoral vessels were seen along dermal fibers. These tumors also showed disorganized small vessels prominently at the tumor edges. In tumors that grew as multiple nodules vascularization was most extensive at the periphery of the individual nodules. Most of the vessels in contact with tumor nodules were of human, not murine origin, especially in melanomas growing in the human reticular dermis. Data will be presented on metastasis formation of melanoma cells from human skin to secondary human skin grafts.

B1-308 GALECTIN-4 EXPRESSION IN HUMAN ADENOCARCINOMAS IS CORRELATED WITH A HIGHLY DIFFERENTIATED PHENOTYPE, M. E. Huflejt[1], A. Krtolica[2], J. W. Ludlow [2,3], H. Leffler [1], S. H. Barondes[1]; [1] Dept. of Psychiatry, Center for Neurobiology and Psychiatry, University of California San Francisco, San Francisco, CA 94143 - 0984; [2]Dept. of Biochemistry; 3 University of Rochester Cancer Center; School of Medicine and Dentistry, University of Rochester, Rochester, NY 14642

Galectin-4 (36 kDa) is a member of a family of β-galactoside-binding lectins, collectively known as galectins. These proteins can be found in a variety of mammalian cell types. Here, we have examined various epithelial carcinoma cell lines for the expression, regulated secretion and localization of galectin-4 in comparison with other galectins. Our results indicate that galectin-4 is abundant in well differentiated polarized human lung, intestinal and ovarian adenocarcinoma cell lines. In contrast, this protein is virtually absent in less differentiated non-polarizing carcinoma cell lines. Galectin-4 is externalized by the well differentiated polarized carcinoma cell lines. Its prominence at the basal membrane of the cells suggests that galectin-4 is involved in cell-substrate adhesion, perhaps contributing to their spreading. Although adenocarcinoma cell lines that are less differentiated and more invasive *in vitro* express little or no galectin-4, they do express other galectins, especially galectin-1.

B1-309 β1-INTEGRINS ARE IMPORTANT FOR TUMOUR CELL INTERACTION WITH HUMAN BONE MARROW STROMA CELLS, Piet Joling and Jan G. Van Den Tweel, Department of Pathology, University Hospital, P.O. Box 85.500, 3508 GA Utrecht, Netherlands.

Tumour cell lines show different binding and migration patterns after contact bone marrow derived stromal cells. The HEL92.1.7 (erythroleukemia) and HL60 (promyeloid leukemia) cells adhere to stromal cells (50 - 70%) showing an enlarged cell contact area (spreading) attenuated by distinct hemidesmosomal contact sites and they invade the stromal monolayer. Some of the HL60 cells are partly retarded at the apical side of the stromal cells, the remaining HL60 cells and all HEL92.1.7 cells cluster underneath the stromal cell monolayer. K562 (CML) cells bind in low numbers (<5%) to stromal cells suggesting the absence contact sites. PC-3 cells and LNCaP cells respectively are bone marrow and lymph node metastases from prostatic carcinoma. PC-3 cells bind immediately to bone marrow stromal cells in contrast to LNCaP cells that show only binding after several hours.

Most of these tumour cells express the ß1-integrins VLA-5, whereas some are positive for VLA-4 and VLA-3. Stromal cells show low expression of VCAM-1, VLA-1 and VLA-5. The function of these adhesion molecules was studied by in vitro inhibition. MAb's directed to CD29 and to fibronectin give an clear inhibitive effect, while also mAb's to VLA-4, VCAM-1 and VLA-5 block interaction to a lesser extent. These data indicate a positive correlation between the expression of VLA-4 and binding of bone marrow derived tumour cells.. Metastasizing cells of prostatic carcinoma bind to stromal cells in absence of VLA-4, pointing to a role of VLA-5 and VLA-3. Blocking with anti-VLA-5 was observed, whereas the role of VLA-3 is further established. Therefore a prominent role of β1-integrins is suggested for normal interaction of bone marrow cells with stromal cells. The same is true for secondary tumour cells.

B1-310 THE METASTATIC POTENTIAL OF THE DUNNING PROSTATE CANCER SERIES EVALUATED ELECTRICALLY Charles R. Keese*, Ivar Giaever*, Alan Partin+, and Donald Coffey+, *Rensselaer Polytechnic Institute and Applied BioPhysics, Inc. Troy, NY; + Johns Hopkins Hospital, Baltimore, MD.

We have used a new, non invasive, analytical measurement for cells in culture to study rat prostatic cancer sublines. In electric cell-substrate impedance sensing (ECIS), cells are cultured on small gold electrodes carrying weak AC signals. The impedance changes of the electrode are used to follow and quantify many complex cell behaviors such as cell spreading and motility in real time (Giaever and Keese, **Nature 366**, 591,1993). Six Dunning sublines were used to determine if ECIS measurements could predict the well documented differing metastatic abilities of the sublines. Electrodes were precoated with laminin or fibronectin before cell attachment. With laminin coated electrodes, all highly metastatic cell lines (AT3, ML, and MLL) demonstrated rapid spreading and increased cell motions compared to their low metastatic counterparts (G, AT1, and AT2). With fibronectin, the low metastatic lines demonstrated greater movement manifested as impedance fluctuations. In related studies, electrodes covered with confluent epithelial cell layers (MDCK) were challenged with Dunning sublines, and the subsequent changes in impedance could also be used to categorize the metastatic abilities of the sublines. The ECIS technique may ultimately allow a dynamic assessment of the metastatic potential of prostate cancer biopsy specimens.

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B1-311 **IN VITRO PENETRATION OF ENDOTHELIAL CELL MONOLAYER AND INVASION OF MATRIGEL BARRIER BY HUMAN LUNG CARCINOMA CELL, Yuhua Li and Cheng Zhu, Bioengineering Center, Georgia Institute of Technology, Atlanta, GA 30332-0405**

As a model to study tumor metastasis, the abilities of a human lung epidermoid carcinoma cell line (Calu-1) to penetrate an endothelial cell monolayer, to invade a matrigel barrier, and to migrate across a transwell filter were tested and compared to those of a human embryonic lung epithelial cell line (L-132) in vitro. A modified Boyden chamber was used, in which confluent monolayers of bovine aortic endothelial cells (BAE) were cultured in some of the transwell filters with 8 μm pores. Other filters were either coated with matrigel or not treated. A radiometric method based on ^{51}Cr labeling was used to quantify the number of cells that transversed the filter and were present in the lower chamber as compared to the total number of cells that were initially added to the upper chamber (expressed as percent of penetration, invasion, or migration). Calu-1 cell showed a significantly ($P < 0.01$ and 0.05, respectively) higher percentage of penetration ($46 \pm 2\%$) across the BAE monolayer and a higher percentage of invasion ($20 \pm 3\%$) through the matrigel barrier as compared to the control L-132 cell ($24 \pm 6\%$ and $15 \pm 1\%$, respectively) after 24 hours of incubation at 37 °C. Calu-1 cell also showed slightly higher percent of migration ($54 \pm 9\%$) than the L-132 cell ($37 \pm 8\%$), although the difference was not statistically significant ($P = 0.055$).

The radiometric measurements were confirmed by direct observations using anti-keratin antibody ABC staining. The dynamic interactions of the Calu-1 cell with the BAE cell monolayer during the penetration process were examined using time-lapse video cinemicroscopy. As the tumor cell invaded underneath the endothelial cell monolayer, clearly observable morphologic changes were seen in the endothelial cells. These included disruption of the cell-cell junction, cytoplasmic vacuolization, endothelial cell retraction, crawling away from the tumor cell and exposing large areas of the underlying surface. In the control experiment, mild morphologic changes were seen in interactions between L-132 cells and the endothelial cell monolayer.

These results show that, in this assay system, the BAE cell monolayer poses less resistance to transmigration of the Calu-1 cell than to that of the L-132 control cell. It is suggested that the response of endothelial cells to factor(s) released by the tumor cell may be responsible for this weakening of the endothelial cell barrier.

B1-312 INVASION OF ACELLULAR, SEMI-PERMEABLE BASEMENT MEMBRANES, Donna Livant,Stephanie Linn, and Jill Shuster, Department of Anatomy and Cell Biology, University of Michigan, Ann Arbor, MI 48109-0616

Sea urchin embryo basement membranes are obtainable intact with both sides recognizable and available for the placement of cells, unlike acellular basement membranes from other sources. Like basement membranes underlying vertebrate epidermis, the inner, basal surfaces of sea urchin embryo basement membranes are permeable to pigment cells as they invade the ectoderm; while their outer surfaces are impermeable. Metastatic melanoma and carcinoma cells invade these basement membranes with high efficiency after placement on their outer surfaces. 15% to 33% of metastatic cells in contact with the basement membranes are found inside after placement on their exteriors and incubation overnight; and the basement membranes appear intact after invasion. Neonatal melanocytes, differentiated keratinocytes, and normal fibroblasts do not cross from the outer to the inner surfaces of these basement membranes. To rule out the crossing of basement membranes by passive migration, we have measured defects in typical preparations of these basement membranes by light and by scanning electron microscopy. We have found that defects larger than 2 microns occur in only 1% of the basement membranes. Since the invading cells are 25 to 30 microns in diameter, defects smaller than 5 microns are very unlikely to admit cells without active invasion. Basement membranes obtained from embryos which failed to gastrulate are crossed by invasive cells with frequencies indistinguishabe from normal basement membranes; thus gut tubes are not significant pathways to their interiors. These results suggest that the ligands eliciting basement membrane recognition and invasion are conserved throughout the evolutionary time separating echinoderms and mammals, and that inappropriate recognition of these ligands may allow metastatic cells to escape their tissues of origin. A rabbit antiserum raised against these basement membranes blocks invasion by more than 10-fold when it is prebound to the basement membrane invasion substrates or to the invading cells, but is not otherwise present in invasion assays. This result suggests that the secretion of basement membrane constituents by invading cells functions in the invasion process itself. *(supported by the Pfeiffer Foundation)*

B1-313 IMMEDIATE EARLY DETECTION OF ENZYMES ASSOCIATED WITH MATRIX DEGRADATION IN REMNANT RAT LIVERS FOLLOWING PARTIAL HEPATECTOMY, Wendy M. Mars, Meng-Lun Liu, William G. Stetler-Stevenson and George K. Michalopoulos, Department of Pathology, University of Pittsburgh, Pittsburgh, PA 15261

Rat livers subjected to a 70% partial hepatectomy (PHx) will regenerate to their normal mass within approximately seven days. One growth factor which has been implicated in the regenerative process is hepatocyte growth factor (HGF), also known as scatter factor. The latent form of HGF can be activated by cleavage with urokinase (uPA) *in vitro*. This phenomenon is also relevant *in vivo* as uPA, bound to its receptor (uPAR), displays enhanced activity in remnant livers within one min following PHx and can cleave single chain HGF to the two chain form. uPA may also activate the matrix metalloproteinase gelatinase A (the 72 kD type IV collagenase or MMP-2). In addition, pretreatment of normal livers with collagenase, followed by infusion of HGF, enhances BrdU labeling to levels found post-PHx. Therefore, we decided to investigate whether enzymes associated with matrix degradation were naturally present in regenerating livers at early time points following PHx. Livers were removed from animals at various time points from one min to 6 hr after PHx. As a control, livers were also harvested at matching time points from sham-operated animals. We now report that in addition to uPAR, significantly enhanced quantities of the matrix-degrading metalloproteinases gelatinase A, gelatinase B (the 92 kD type IV collagenase or MMP-9), and interstitial collagenase (MMP-1) are present in remnant livers one min after PHx. In contrast, the metalloproteinase inhibitor TIMP-2 is not significantly different in the sham-operated and hepatectomized livers. Western blot analysis indicates the active 62 kD form of gelatinase A appears at five min after surgical resection. Thus, degradation of extracellular matrix is apparently an early event that may modulate liver regeneration.

B1-314 Enhanced Expression of the Gene for Osteopontin in Metastatic Rat Mammary Epithelial-Derived Cell Lines in Comparison to their Non-Metastatic Parent, Adam J. Oates, Roger Barraclough and Philip S. Rudland, Cancer and Polio Research Fund Laboratories, Crown St., Liverpool, L69 3BX. U.K.

The rat mammary epithelial cell line, Rama 37, yields benign, non-metastasizing adenomatous tumours in syngeneic Wistar-Furth rats. Following transfection of this stably diploid cell line with genomic DNA fragments from a human metastasizing breast cancer cell line, a number of animals, when injected subcutaneously with the newly transfected cells, develop secondary tumours. From one such secondary lung tumour, a cell line was established designated Ca2-5-LT1, which, when introduced into the host, also showed the ability to metastasize. A general method of subtractive hybridisation has been used for the identification of genes expressed differentially in the metastatic Ca2-5-LT1 and the parental benign Rama 37 cell lines. So far out of three differentially expressed cDNA clones, one clone has a 9-11 fold higher level of specific mRNA in the metastatic line than in the nonmetastatic parental Rama 37 cell line. This cDNA when sequenced was found to correspond to the mRNA for rat osteopontin, a secreted phosphoprotein, previously implicated in metastasis in other systems. The levels of mRNA for osteopontin in other malignant, metastatic mammary cell lines, produced spontaneously (Rama 800) and by transfection of DNA from metastatic human mammary cell lines (Ca2-T7-LNT1, Ca2-T3-LT1), were also increased dramatically, whilst a metastatic cell line produced by transfection of the metastagene for p9Ka failed to show elevated levels of this mRNA. It is suggested that osteopontin is another metastagene in this rat mammary system acting differently from p9Ka.

B1-315 REGULATION OF LFA-1-MEDIATED ADHESION IN LYMPHOMA METASTASIS, Ed Roos, Mariëtte H.E. Driessens, Paula van Run, Ellen van Rijthoven and Geertje La Rivière, Division of Cell Biology, The Netherlands Cancer Institute, 121 Plesmanlaan, 1066 CX Amsterdam.

Invasion and metastasis of T-cell hybridomas are completely dependent on expression of the adhesion molecule LFA-1. This LFA-1 is not constitutively active, but becomes activated during interactions with monolayers *in vitro* and apparently during invasion of tissues *in vivo*. Pertussis toxin, which strongly inhibits invasion and metastasis, blocks this activation. Previously, we have generated LFA-1-deficient mutants of the TAM2D2 T-cell hybridoma. Independent mutants, deficient in either the α- or the β-chain of LFA-1, had virtually lost invasive and metastatic capacity. However, transfection of the α- or the β-chain cDNA into these mutants did not restore invasiveness, whereas spontaneous revertants with similar LFA-1 levels did reacquire their invasive potential. We have now generated a monoclonal antibody against a surface protein that, in addition to LFA-1, is missing in one of the mutants. The level of this protein is, in contrast to LFA-1, also low on non-invasive T-cell hybridomas. This antibody reduced adhesion of the cells to substrates coated with the purified LFA-1 counterstructure ICAM-1. We have no evidence yet for a physical association between the protein and LFA-1, since LFA-1 is not co-precipated by the monoclonal antibody. We conclude that this protein is involved in LFA-1 function, possibly in the regulation of LFA-1 activity, and its absence in one of the mutants may explain why LFA-1 transfection did not restore invasiveness in that mutant. Furthermore, the lack of invasion of the non-invasive hybridomas may perhaps be attributed to the low expression level of this protein. The characterization of this protein and its functional effects are in progress and we hope to present additional data at the meeting.

B1-316 EGF STIMULATION OF RAT MAMMARY ADENOCARCINOMA CELLS INDUCES ACTIN REORGANIZATION, Jeffrey E. Segall*, William Miroff*, Lucy Boselli+, Amanda Chan*, Sangeeta Tyrech+, Joan G. Jones#, and John Condeelis*, Departments of *Anatomy and Structural Biology, #Pathology and +Radiation Oncology, Albert Einstein College of Medicine, Bronx, NY 10461

Metastasis is proposed to depend on a number of processes including cell adhesion, pseudopod extension, and motility in response to specific cytokines. Dynamic image analysis of video recordings was performed on a variety of tumor cell lines and cytokines to determine which cell line/cytokine pairs demonstrated the most dramatic response to stimulation. The rat mammary adenocarcinoma cell line MTLn3 showed a significant shape change in response to EGF, a growth factor found in normal breast tissue. Further analysis indicated that the shape change produced by sudden uniform increases in EGF is caused by an increase in cell area due to lamellipod extension. The mean cell area increases by about 30%, peaking at 3-4 minutes after stimulation, and the K_{50} for this response is 0.5 nM EGF, within the range of K_D values of EGF for its receptors. Analysis of cell migration demonstrates that peak migration occurs at 5 nM EGF and is primarily a chemotactic response. Confocal microscopy of cells demonstrates that newly formed lamellipods contain increased concentrations of F-actin under the leading lamellar membrane. Three minutes after stimulation, NBD-phallacidin binding assays indicate little change in the total cellular F-actin content. Cytochalasin D, at concentrations which inhibit actin polymerization, inhibits the EGF-induced increase in area and lamellar extension. These results indicate that EGF-induced lamellipod extension involves a cytochalasin D-sensitive actin polymerization step. We propose that this polymerization leads to the accumulation of F-actin observed at the leading edge of EGF-elicited lamellipods.

B1-317 MESENCHYME ACTIVATION BY EPITHELIAL TUMOR CELLS IN A NOVEL TUMOR INVASION AND ANGIOGENESIS MODEL, Mihaela Skobe and Norbert E. Fusenig, Division of Differentiation and Carcinogenesis, German Cancer Research Center, 69120 Heidelberg, Germany

Invasive growth of carcinoma cells implies an altered epithelial-mesenchymal interaction. To study this interaction in more detail, we have developed an in vivo transplantation assay where immortalized human skin keratinocytes (HaCaT) of different tumor stages are grown as a surface epithelium on a collagen gel type I and their interaction with the underlying mesenchyme analyzed during preinvasive and invasive stages. We demonstrate that, depending on the stage of transformation, keratinocytes exhibit individual pattern of mesenchyme activation. Malignant cells were found to differ from benign and non-tumorigenic cells by their capacity to generate a strong and directed ingrowth of blood vessels as early as four days after transplantation, and to maintain a high degree of vascularization in the stroma. In contrast, during normal wound healing process (transplants of collagen type I alone or with HaCaT cells) and transplants of benign cells, angiogenesis was never directed and blood vessel sprouting ceased after about two weeks. Since in transplants of malignant keratinocytes cells were, prior to invasion, separated from the mesenchyme by a solid collagen gel, diffusible factors responsible for differences in angiogenic response seemed to be also crucial for invasion. Vascular Endothelial Growth Factor (VEGF) is thought to play a major role in tumor angiogenesis. To elicit its role in induction and/or maintenance of tumor angiogenesis and its significance for tumor invasion we then analyzed the expression of VEGF and its high affinity receptor (FLK-1) by in situ hybridization. VEGF is expressed in all cell lines during initial angiogenesis induction, but is upregulated only in well formed tumors. Strong FLK-1 expression is restricted to endothelium activated by malignant cells and could be demonstrated at both very early and late stage of tumor growth. These results suggest that VEGF is involved in maintenance of tumor angiogenesis, while its role for its early induction and tumor cell invasion is not yet clear.

B1-318 CLONING OF NOVEL CELL SURFACE ADHESION MOLECULES FROM MALIGNANT MELANOMA AND NEUROBLASTOMA: EVALUATION OF THEIR ROLE IN TUMOUR PROGRESSION

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Tumour progression is usually associated with an increase in the malignant potential of tumour cells, which correlates with an increased incidence of tissue invasion and metastases, and a worsening of prognosis. In some tumours, the malignant potential increases markedly at a well-defined stage of development, as in the neural crest derived tumours malignant melanoma (MM) and neuroblastoma (NBL). In MM, this occurs during the radial-to-vertical growth phase transition, whereas in NBL it occurs during the transition from stage I/II to III/IV. One of the key factors thought to be involved in this transition are the cell surface adhesion molecules (CSAMs). These include cadherins (cell-to-cell interactions), integrins (cell-to-matrix interactions), and selectins (migration into and out of blood vessels). By identifying the CSAMs involved in cell invasion and motility in these tumours, we hope to i) obtain a better understanding of the biological factors involved in these tumours and ii) identify possible targets for improved diagnostic and therapeutic interventions.

To better define the role of CSAMs in the progression of these tumours, we have made cDNA libraries from many MM and NBL tumour samples. The products of degenerate primer PCR (using primers to various CSAMs) have been cloned and sequenced. Database analysis has shown that many of the cloned fragments share sequence homology to CSAMs, including integrins, cadherins, and selectins. Cellular signalling molecules that may be involved in increasing the malignant potential of these tumours have also been considered, and a putative novel G-protein-coupled receptor and G-protein associated kinase, isolated from NBL, will be described. These clones and their homologues isolated from normal human tissue will be used to examine MM and NBL tumour samples of differing grades of malignancy for localisation of expression and abnormalities of expression and structure.

B1-319 INHIBITION OF PKC INDUCES MOTILITY IN DIVERSE LYMPHOCYTE POPULATIONS

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Circulating lymphocytes are rounded, non-motile cells which must acquire a constantly shape-changing, polarized morphology to facilitate migration into appropriate sites. Here we show that bisindolylmaleimide inhibitors of PKC (GF 109203X, Ro 31-8220, CGP 41 251) induce lymphocytes to change rapidly (<30 min) into polarized, locomoting cells. This effect was seen with T cells, tonsillar B cells and Jurkat and MOLT-4 T cell lines. Bisindolylmaleimide-induced motility was prevented by pretreatment with the phosphatase inhibitor calyculin A. Chronic treatment (44h) with bryostatin also induced motility in PBL and a constitutively non-motile subline of MOLT-4 cells. This correlated with the downregulation of PKC-α and -β by more than 95% (and PKC-ϵ and -zeta to a much lesser extent). In contrast, treatment of a spontaneously motile subline of MOLT-4 cells with various PKC activators caused a rapid reversion to a rounded morphology. The activity of a PKC or related enzyme may therefore maintain a rounded morphology in resting lymphocytes. Alterations in the balance between kinase/phosphatase activity may provide a switch for the transition to a motile phenotype.

B1-320 NOVEL CELL SURFACE ADHESION-SIGNALLING MOLECULES EXPRESSED DURING NEURAL CREST CELL MIGRATION : EVALUATION OF THEIR ROLE IN CANCER CELL INVASION AND MOTILITY IN MELANOMAS AND NEUROBLASTOMAS.

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Cancer progression through tissue invasion to metastasis can be associated with a dramatic worsening of the prognosis, as exemplified in neural crest (NC) tumours by the radial to vertical phase transition in melanomas (MM), and progression from stage II to Stage IV in neuroblastomas (NBL). Factors mediating the processes of invasion and metastasis need to be identified for diagnostic and prognostic purposes and may represent possible targets for therapy, particularly if the process can be inhibited or reversed. Several classes of cell surface adhesion molecules (CSAM's) are believed to contribute towards invasion and metastasis, although few have been defined in NC tumours. Our approach is based on evidence indicating that i) neoplasms can arise due to dysfunction of genes (including CSAM's) involved in normal embryogenesis and cell differentiation, and that ii) many of these processes are highly conserved throughout evolution. Accordingly we have made cDNA libraries representing genes expressed during the phases of avian embryonic melanocyte and neuroblast precursor cell development. The libraries have been used to derive further "sub-libraries" (by degenerate primer PCR), using primers to various CSAM's (including cadherins, Ig superfamily, integrins and N-CAM), as well as receptor tyrosine kinase and phosphatase sequences. Additional sub-libraries designed to yield novel adhesion-signalling molecules have been generated by amplifying across the putative transmembrane region using varying combinations of adhesion and kinase / phosphatase primers. We will describe cloned fragments that appear to be novel CSAM's, as determined by database analysis. The human cognate clones will be used to examine our large collection of melanomas and neuroblastomas of various clinical and biological stages for structural abnormalities, abnormalities of expression, or expression confined to the critical biological stages of tumour progression.

B1-321 ACTIVATED RAC1 IS ONCOGENIC AND INCREASES CELL LOCOMOTION IN RAT1 FIBROBLASTS, Marc Symons, Rong-Guo Qiu and Frank McCormick, Onyx Pharmaceuticals, Richmond, CA 94806

In order to study the role of the small GTP-binding protein rac1 in the control of cell proliferation and cell motility, we have established Rat1 fibroblast lines overexpressing various mutants of rac1 driven by a tetracycline-responsive promotor. We found that lines overexpressing rac1V12, a constitutively activated mutant, grew to higher saturation density and showed enhanced growth in low serum with respect to vector controls. These lines also proliferated in soft agar and induced tumors in nude mice. In contrast, cells overexpressing rac1N17, a dominant negative mutant, grew to a lower saturation density, displayed a slower growth rate and did not grow in soft agar. Thus, rac1 controls cell proliferation and overexpression of rac1V12 is oncogenic. With regard to the organization of the cytoskeleton, the rac1V12 lines showed constitutive lamellipodia formation, even in the absence of serum or upon reaching confluence. In addition, rac1V12 lines also showed a higher motility rate in monolayer wounding assays than vector controls, suggesting a possible link between the production of lamellipodia and cell motility.

B1-322 ROLE OF MATRIX METALLOPROTEINASES IN THE FORMATION OF VASCULAR TUMORS AND IN ANGIOGENESIS INDUCED BY ENDOTHELIOMA CELLS G. Taraboletti, D. Belotti, A. Garofalo, T. Drudis, P. Borsotti, P.D. Brown[1], and R. Giavazzi. Istituto di Ricerche Farmacologiche Mario Negri, 24125 Bergamo, Italy and [1]British Biotech Ltd., Cowley, Oxford OX4 5LY, England.

Endothelioma cells transformed by polyoma virus middle T oncogene represent a unique model to study tumor associated angiogenesis and vascular tumors. In vivo they form hemangioma-like tumors, constituted by more than 95% of host recruited cells; in vitro they release a factor (named EDMF-endothelioma derived motility factor), which recruits endothelial cells (Cancer Res 53: 3812-3816, 1993). The role of metalloproteinases in cell invasion and angiogenesis is well documented. We have investigated the involvement of metalloproteinases in endothelioma cell growth in vivo and in endothelial cell recruitment using a synthetic matrix metalloproteinases inhibitor, batimastat (BB94). The effect of batimastat was studied on tumor formation by endothelioma cells injected subcutaneously in nude mice. Daily treatment with batimastat (30-0.3 mg/Kg at the site of tumor cell injection) inhibited tumor growth, with a significant increase in doubling time and animal survival. An analog of batimastat, BB374, less active than batimastat in inhibiting metalloproteinases, resulted also less active in reducing hemangioma growth. In vitro, batimastat did not affect endothelioma and endothelial cell proliferation, nor endothelial cell motility in response to EDMF, but it significantly inhibited endothelial cell invasion through a reconstituted basement membrane (Matrigel). These findings indicate that batimastat reduces the growth of experimental vascular lesions, most probably by blocking endothelial cell recruitment by the transformed cells, and suggest a therapeutic potential for metalloproteinases inhibitors in angiogenesis and vascular tumors.

B1-323 FIBRONECTIN INTERMEDIATES TUMOUR CELL ADHESION TO HUMAN BONE MARROW STROMAL CELLS, Jan G. Van Den Tweel and Piet Joling, Department of Pathology, University Hospital, P.O. Box 85.500, 3508 GA Utrecht, Netherlands.

Human bone marrow stromal cells show a prominent production of fibronectin. Cultures show intracellular production of fibronectin within an hour and extensive extracellular fibronectin is detected within hours. Fibronectin is important for cell interactions in the bone marrow. The promyelocyte leukemia line HL60 and the erythro leukemia line HEL92.1.7 show intensive binding to stromal cells whereas low binding of K562 (CML-line) cells is seen. Tumour cells from other organs, such as the prostate tumour metastasis line PC-3 (bone marrow) bind stromal cells but LNCaP (lymph node), shows no clear binding. The cell interactions between stromal and tumour cells can be inhibited by polyclonal anti-fibronectin antibody, indicating to an essential role of fibronectin. Since the ß1-integrins VLA-3, VLA-4 and VLA-5 are described as ligands of fibronectin, the expression of these adhesion molecules was defined on tumour cells and stromal cells. HEL92.1.7 cells highly express VLA-4, while a lower expression is detected for HL60 cells. PC-3 is negative for this integrin. VLA-3 is positive on PC-3 and HEL92.1.7 cells, whereas a low expression is seen on HL60 cells. VLA-5 is detected on all tumour cell types, but also bone marrow derived stromal cells express VLA-5.

MAb's directed against these integrins show variable inhibitive effects on the interaction of stromal cells and the tumour cells. Adherence of tumour cells was further studied using solid phase fibronectin. These experiments suggest a prominent role of fibronectin in tumour cell adhesion with different ligands. VLA-4 seems important for tumour cells derived from bone marrow cells, whereas localisation of secondary tumour cells seems to be controlled by VLA-5 or VLA-3.

B1-324 A DUAL FUNCTIONAL SIGNAL MEDIATOR SHOWING RhoGAP AND PHOSPHOLIPASE C-δ-STIMULATING ACTIVITIES, Yoshimi Homma and Yasufumi Emori, Department of Biosignal Research, Tokyo Metropolitan Institute of Gerontology, Itabashi-ku, Tokyo 173, and Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Phosphatidylinositol 4,5-bisphosphate (PIP_2) is a minor component of membrane phospholipids and functions as a precurcer of intracellular second messengers. In addition to these roles, another function of PIP_2 has been proposed since a number of PIP_2-binding proteins, such as gelsolin, cofilin, profilin, and a-actinin, have been found. These PIP_2-binding proteins are known to bind to actin and regulate various cell functions in which cytoskeletal organization is involved, such as cytokinesis and cell motility. We have cloned a novel regulator protein, p122, in the Rho-signalling pathway by screening a rat brain expression library with antiserum raised against purified phospholipase C-δ1 (PLC-δ1). As suggested by the deduced amino acid sequence, this regulator protein shows a similarity to the GTPase-activating protein (GAP) homology region of Bcr and possesses GAP activity for RhoA, but not for Rac1; no guanine nucleotide-exchange activity for RhoA and Rac1 was detected. This novel p122-RhoGAP binds to PLC-δ1 and activates the PIP_2 hydrolyzing activity of PLC-δ1. These findings suggest that this novel RhoGAP is involved in the Rho-signalling pathway, probably downstream of Rho activation, and mediates the stimulation of PLC-δ, which leads to actin-related cytoskeletal changes through the hydrolysis of PIP_2 which binds to actin-binding proteins such as gelsolin and profilin. Further biochemical and cell biological studies should address involvement of this novel dual function protein in tumor metastasis.

Paracrine Motility and Growth Factors;
New Therapies and Diagnostic Applications

B1-400 FOCAL ADHESION KINASE IN ENDOTHELIAL CELLS. R. Alessandro, J. Spoonster, and E. C. Kohn. Signal Transduction and Prevention Unit, Laboratory of Pathology, National Cancer Institute, Bethesda, MD 20892

Angiogenesis is a tightly regulated phenomenon that may be stimulated in response to soluble factors or components of the extracellular matrix (ECM). The molecular basis of ECM-induced angiogenesis is not well characterized, but may be triggered by endothelial cell contact with the ECM at sites known as focal adhesions. Focal adhesion kinase (pp125FAK) is a 125 kDa protein tyrosine kinase which may link the signals generated at the cell membrane by cytoskeleton-associated processes. Tyrosine phosphorylation is a key step in the activity of pp125FAK. Little is known about the role of pp125FAK in angiogenesis. We and others have demonstrated tyrosine phosphorylation of MR 110-150 kDa proteins in human umbilical vein endothelial cells (HUVEC). This study investigated pp125FAK during the adhesion of HUVECs to ECM. HUVEC adhesion to type IV collagen resulted in tyrosine phosphorylation of pp125FAK as detected by immunoprecipitation and western blot; little or no phosphorylation was observed when cells were exposed to control of type IV collagen in suspension and little tyrosine phosphorylation was seen when cells were adhered to tissue culture plastic. At 10 min, when HUVECs were fully adherent but not yet spread on collagen type IV, an increase in tyrosine phosphorylation of pp125FAK was observed. At 45 min when HUVECs were in the process of spreading, pp125FAK tyrosine phosphorylation was maximal, several-fold over that seen for tissue culture plastic exposure. The highest level of pp125FAK tyrosine phosphorylation was observed before the HUVECs were completely spread. Exposure of HUVECs to laminin and fibronectin in solution resulted in no phosphorylation of pp125FAK, whereas exposure and adhesion of HUVECs to laminin- and fibronectin-coated dishes caused marked tyrosine phosphorylation of pp125FAK, but not to the level seen with adhesion to type IV collagen. These data suggest that pp125FAK tyrosine phosphorylation may be activated in HUVECs during adhesion to ECM and that it is further augmented during the process of spreading.

B1-401 IL-2R EXPRESSION ON NON-LYMPHOID TUMOR CELLS CORRELATES WITH INCREASED METASTATIC POTENTIAL, Emilio Barberá-Guillem and Sulekha Rao, Hipple Cancer Research Center, Dayton, OH 45439

The receptor for Interleukin-2 (IL-2R) is expressed mainly on lymphoid cells which respond to Interleukin-2 (IL-2) as a growth factor. It has been reported that IL-2 deficiency as a consequence of T cell anergy is the main factor involved in the failure of the immune system against tumor cells. We have observed that a very high metastatic line of B16F10 murine melanoma cells spontaneously express IL-2R. Both the Northern blot and PCR analysis revealed the expression of IL-2Rα mRNA in B16F10 cells. A single transcript of 1.9 kb was found in these cells using mIL-2Rα cDNA as a hybridization probe in Northern blot analysis. Western blot analysis showed a single band of 55 kDa. We further investigated the functional role of IL-2R and the correlation of its expression on tumor cells with their metastatic potential. We observed that cell cultures in nonadherent conditions upregulated IL-2R expression. The addition of IL-1, IL-2 and IL-6 *in vitro* also modulates the expression of IL-2Rα and IL-2Rβ on these melanoma cells. The expression of the IL-2Rα was detected by immunofluorescence on the surface of 10-30% of the tumor cells, in direct correlation with the mRNA expression. Moreover, in more than 50-80% of these cells, internal IL-2Rα was detected by immunofluorescence after cell permeabilization. Low concentrations (1-10 IU/ml) of exogenous IL-2 increase B16F10 growth in serum free medium. In fibrin clot cultures, similar doses of IL-2 increased colony formation by 50%. These B16F10 cells also transcribed IL-2 mRNA, but did not secrete detectable amounts of IL-2 *in vitro*. Low concentrations of IL-1β increased IL-2 and IL-2Rα mRNA expression on these cells *in vitro*. IL-1β added to culture in serum free conditions induced B16F10 cell proliferation. This result was blocked by anti-IL-2Rα antibody, showing that the growth stimulating effect of IL-1β is mediated by autocrine IL-2. *In vivo* experiments showed that the number and volume of liver metastasis obtained after splenic injection of B16F10 cells directly correlated with the number of injected cells expressing IL-2Rα. To test the pro-metastatic role of IL-2R expression on tumor cells, B16F10 cells were co-transfected with two vectors carrying IL-2Rα and the neomycin resistance genes, respectively. B16F10 cells containing 10% IL-2Rα transfected cells and 90% neomycin transfected cells were injected into the spleens of five mice. After 14 days, 15% of the B16F10 cells obtained from the splenic tumors hyper-expressed IL-2Rα whereas 75% of the cells obtained from the liver metastasis hyper-expressed IL-2Rα. These results show that the tumor cells expressing IL-2Rα are positively selected for liver metastasis. Taken together, these findings suggest that tumor cells expressing IL-2R are better able to evade immunosurveillance and develop metastases.

B1-402 ANTI-METASTATIC PROSTACYCLINS INHIBIT E-SELECTIN MEDIATED ADHESION OF COLON CARCINOMA TO ENDOTHELIAL CELLS. George Daneker, Serena Lund, Wright Caughman, and William Wood, Department of Surgery, Emory University Atlanta, GA 30322

Introduction: Arachidonic acid metabolites, especially prostacyclin, have long been shown to have anti-metastatic activity. One explanation is their modulation of adhesion molecule (CAM) expression by target organ endothelial cells. We have postulated that prostacyclin, and it's mechanistic mimics, decrease colon carcinoma adhesion to cytokine stimulated endothelial cells by blocking expression of E-selectin.

Methods: Cultured human microvascular endothelial cells (MEC) were pre-incubated with prostacyclin (PGI), forskolin, iso-methylbutylxanthine (IBMX), or dibutyrl-cAMP for 15 minutes, then co-incubated with the cytokine TNF for 6 hours. MEC surface expression of E-selectin was evaluated by ELISA. Adherence of ^{51}Cr labeled COLO 205 cells to MEC monolayers was determined. In parallel, MECs were incubated with E-selectin monoclonal antibody (1:500) prior to the addition of tumor cells. Data is presented as mean ± SEM, * $p < 0.05$ by single factor ANOVA.

Results: *Fig. 1* Prostacyclins significantly reduced E-selectin expression by MEC. *Fig. 2* Prostacyclins significantly decreased colon carcinoma adherence to stimulated MECs. In general, the inhibition of E-selectin expression corresponded to the reduction of tumor cell adherence. Prostacyclin's effects on tumor adhesion was negated by pre-incubation with E-selectin antibody (Control and PGI shown).

Fig. 1 E-Selectin ELISA

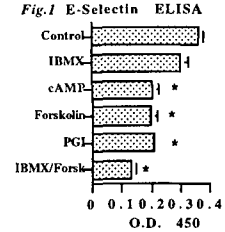
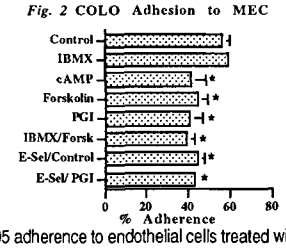


Fig. 2 COLO Adhesion to MEC



Conclusions: The inhibition of COLO 205 adherence to endothelial cells treated with prostacyclin and it's mimics appears to result from blocking endothelial E-selectin expression. These data support the hypothesis that prostacyclins may exert their anti-metastatic effect, in part, by inhibiting CAM mediated adherence of colon carcinoma to endothelial cells in target organs.

B1-403 TARGETED DISRUPTION OF BOTH CD44 ALLELES IN A LYMPHOMA CELL LINE TO STUDY THE ROLE OF CD44 IN METASTASIS

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CD44 is a widely expressed cell surface molecule involved in extracellular matrix binding, cell migration, lymphopoiesis and tumor growth and metastasis. Alternatively spliced forms of CD44 have been implicated in metastasis of carcinomas. In addition, expression levels of the standard CD44 molecule, lacking the variable domains, appear to correlate with tumorigenicity and metastatic capacity of lymphomas and melanomas. To study this, we have sequentially disrupted both alleles of the CD44 gene in the highly metastatic lymphoma MDAY-D2 by homologous recombination. The metastatic capacity of the CD44-negative mutants is presently being investigated and the results will be presented at the meeting.

B1-404 EXPRESSION OF THROMBOSPONDIN (TSP-1) DURI[illegible] DEVELOPMENT OF THE HUMAN MAMMARY GLAND AND IN *IN SITU* A[illegible] INVASIVE CARCINOMA OF THE BREAST. L. Frappart, Z. Abbadia, N. Bertin, [illegible]. Clezardin. Pathology, Bât 10 and INSERM U 403, Pavillon F, Hôpital Edouard Herriot, F 69437 Lyon Cedex 03.

Thrombospondin (TSP) is a trimeric extracellular matrix glycoprotein involved in embryogenesis and organogenesis. Five genes encoding for four distinct TSPs (TSP1, TSP2, TSP3, TSP4), and cartilage oligomeric matrix protein (COMP) have recently been described. In this study, the patterns of expression of TSP1 and of two known cell surface receptors: CD36 and CD51 have been determined during development of the human fetal mammary gland and in normal, hyperplastic and neoplastic human breast.TSP1 was found in the dense mesenchyme immediately adjacent to the mammary bud, and at the membrane of budding epithelial cells invading the surrounding mesenchyme. As formation of the ductal tree system occurs, TSP1 becomes expressed at the myoepithelial-stromal junction of mammary ducts. Such an immunolocalization of TSP1 has been confirmed at the mRNA level by *in situ* hybridization and also by polymerase chain reaction assay. Comparison of TSP1 immunolocalization with that of CD36 and CD51, revealed no colocalization. As opposed to TSP1, CD36 was strongly expressed at the membrane of preadipocytes present in the fat pad tissue, but absent from epithelial cells of the mammary bud. CD51 was only weakly expressed by budding mammary epithelial cells and did not colocalize with TSP1. In nonlactating ducts of normal and hyperplastic breast, TSP1 and CD51 are expressed in the basement membrane and in the basal surface of myoepithelial cells, respectively. In lactating adenomas, both TSP1 and CD51 disappear from the myoepithelial-stromal junction of the ducts. However, TSP1 becomes selectively expressed at the apices of secretory epithelial cells of lactating ducts together with CD36. In neoplastic human breast, a strong immunoreactivity for TSP1 is observed in the basement membrane surrounding *in situ* carcinomas and excessive TSP1 deposits are observed in desmoplasia of invasive ductal carcinomas and in fibroblast present in desmoplastic areas. On the other hand, few invasive ductal carcinoma cells express TSP1, while CD51 is moderately expressed by some neoplastic clusters. Moreover, we have studied the TSP1 expression in human breast cancer with quantitative reverse transcriptase-polymerase reaction (RT-PCR).The first results show a correlation between TSP1 and the tumor diameter, but no correlation with the lymph-node status and the other prognostic factors. Taken together, these findings suggest that TSP1 plays an important role during development and differentiation of the human mammary gland and in carcinomas of the breast.

B1-405 MODULATION of METASTATIC BEHAVIOR by TUMOR pH. Robert J. Gillies, Raul Martinez-Zaguilan, EliSabeth Seftor and Mary JC Hendrix. Dept. Biochem., Univ. Arizona HSC, Tucson, AZ 85724; and Pediatric Research Inst., Cardinal Glennon Hosp., St. Louis Univ., St. Louis, MO. 63110

For years, it has been hypothesized that tumors were more acidic than normal tissues. Recently, we have developed an *in vivo* ^{31}P NMR based technique to measure the extracellular pH of RIF-1 and MCF-7 tumors and have determined that the pH of tumors is approximately 6.7, as compared to 7.1 for more normal tissues. These results are in close agreement with the more invasive microelectrode measurements.

We have examined the effect of this acidic pH on the invasive behavior of human breast cancer and human melanoma cells in culture using Membrane Invasion Culture System (MICS) chambers. We have observed that acid pH significantly stimulates invasive behavior, a key step in metastasis. Metastatic behavior was tested *in vitro* using . In both C8161 and A375P melanoma cells, the stimulatory effect of pH on migration and invasion is not due to a direct trophic effect of pH on the metastatic machinery, since the effects were only observed following prolonged exposure to acidic conditions. In MCF/7 breast cancer cells, the effect of pH is observed only under acute conditions. These differences may relate to differences in proteases for these two cell lines.

We have also observed that, although there are no steady-state differences in the intracellular pH, more invasive melanoma and breast cancer cells express a novel pH regulatory mechanism: i.e. plasma-lemmal Vacuolar-type H^+-ATPase (V-ATPase) activity. However, induction of V-ATPase activity does not stimulate migration or invasion, suggesting that V-ATPase activity and metastasis are not functionally coupled. Supported by NIH grants GM43046 (RJG) and CA59702 (MJCH), ACS grant No. CN-53 (RJG).

B1-406 PRELIMINARY RESULTS OF A PHASE I TRIAL OF CM101 IN CANCER PATIENTS. C.G. Hellerqvist, G.B. Thurman, B.D. Wamil, R.F. DeVore, H.W. Sundell, D.L. Page, M. Zhang, C. Carter, H. Yan, G.B. Wakefield, and D.H. Johnson, Depts. of Biochemistry, Pediatrics, Pathology, Medicine and Biology, Vanderbilt University, Nashville, TN 37232-0146.

Group B Streptococcus (GBS), isolated from human neonates diagnosed with sepsis and respiratory distress, produces a polysaccharide exotoxin (CM101) which we have previously described as GBS Toxin[1]. CM101 is thought to be the toxin responsible for the pulmonary pathophysiology associated with neonatal GBS pneumonia. We hypothesized that CM101 bound to developing endothelium in the neonatal lung and likewise could bind to neovasculature in developing human tumors. CM101 inhibits tumor growth[2] and causes tumor ablation[3] through induction of an inflammatory response focused on tumor neovasculature in mice. In Phase I trial, CM101 has been infused in saline i.v. over a 15 min. period qMWF at 7.5-25μg/kg for one week. CM101 induced, as anticipated, acute inflammation with grade 1-2 fever and chills in most cases. Changes in white cell counts were indicative of induced acute inflammation. Circulating lymphocytes decreased as much as 80% and monocytes as much as 100% and stayed low for up to 8 hrs. Biopsy of an adenocarcinoma following CM101 treatment showed evidence of sequestering of WBC and inflammatory reaction within the tumor tissue also seen in our mouse model. CM101 has been demonstrated not to effect physiologic neovascularization wound healing[4].

CM101 induces a dose-dependent systemic cytokine cascade of MIP-1α, TNF , IL-6, IL-8 and IL-10. Elevated systemic sE-Selectin demonstrated engagement of endothelium in the inflammatory process. Twelve patients with various tumor types have been treated with CM101 with qualitatively similar cytokine and sE-Selectin response patterns. Tumor responses were observed in three patients (duodenal adenocarcinoma, Kaposi sarcoma, and hepatocellular carcinoma). One patient (renal cell cancer) had tumor stabilization. No delayed side effects or significant immunity to CM101 have been observed after as many as five repeat cycles. (Supported by CarboMed, Inc.)

[1]Ped. Res. 15:892 (1981); [2]Canc. Res. Clin. Oncol. 120:63-70 (1993); [3]Ibid., 120:479-484 (1994); [4]In preparation (1994).

B1-407 A NEUTRALIZING ANTI-HEREGULIN ANTIBODY INHIBITS THE INVASIVE BEHAVIOR OF BREAST CANCER CELLS, MDA-MB-231, AND INDUCES PRODUCTION OF ALPHA-LACTALBUMIN. M.M. Hijazi and R. Lupu, Vincent T. Lombardi Cancer Research Center, Georgetown University Medical Center, 3800 Reservoir Rd. NW, Washington D.C. 20007.

The metastatic process comprises changes in tumor cell adhesion properties, cell motility and remodeling of the extracellular matrix. Previous data from our laboratory suggest that the *erbB* ligand heregulin may be a significant factor in the induction of the invasive phenotype in heregulin stimulated SKBR-3 cells. MDA-MB 231 cells produce high levels of heregulin and are classified amongst the most invasive breast cancer epithelial cell lines. MDA-MB-231 cells are extremely invasive and chemotactic cells, as determined by the Boyden Chamber assay. Within a 6 hr incubation period migration was blocked by 40-80% when cells were treated with increasing concentrations of the anti-*heregulin* antibody (20-80 ug/ml). After treatments with the neutralizing antibody for 7 days, a marked growth inhibitory effect as well as inhibition of the outgrowth assay in Matrigel was observed. These effects were accompanied by profound morphological changes characteristic of breast cancer cell differentiation. This was represented by an increase in size and a more elongated appearance, as determined by staining for F-actin with phalloidin. Production of alpha-lactalbumin was apparent after 4 days of incubation with the anti-heregulin antibody (40-80 ug/ml) demonstrated by immunostaining with a specific anti-human alpha lactalbumin antibody. No staining was present in untreated MB-MDA 231 cells. This data supports a potential role for application of the heregulin neutralizing antibody in breast cancer therapy.

B1-408 sCD44var(v6) - IS IT A RELIABLE TUMOR MARKER? Marianne Jochum[1], D. Inthorn[2], M. Schorr[1], Ch. Waydhas[2], I. Weichselbraun[3] and H. Fritz[1], Devision of Clinical Biochemistry[1] and Departments of Surgery[2] at the University Munich, Germany; Bender Med Systems[3], Vienna, Austria.

In contrast to the nearly ubiquitously expressed standard form of the adhesion molecule CD44 splice variants (CD44var) have a much more restricted distribution, e.g. exon v6 on activated lymphocytes and macrophages as well as on primary and metastatic human tumors. As shown recently by immunohistochemical findings CD44var(v6) seems to play an important role during tumor progression and formation of metastases. Since a soluble form of this adhesion molecule can be detected in human body fluids with a specifically designed ELISA (Bender MedSystems, Vienna) the aim of this study was to show whether sCD44 var (v6) is elevated in serum of tumor patients above levels measured in healthy controls or patients suffering from sepsis or multiple trauma. A mean value of 101 ng/ml was measured in 10 healthy controls and of 89 ng/ml in 14 tumors patients (mainly colorectal tumors with tumor stages T3/T4 and metastases) prior to selective surgery. Follow-up samples in patients developing sepsis after selective tumor surgery showed normal serum levels in 2 patients, levels between 100 and 200 ng/ml in another 2 patients, and elevations up to 480 ng/ml in 2 additional patients. In 2 non-tumor patients with sepsis due to a bilio-pulmonary fistula or aspiration pneumonia serum levels between 200 and 300 ng/ml were measured. A correlation between CD44var(v6) levels and the severity of the inflammation was not observed in any of the septic patients. In trauma patients, however, the increasing serum levels from normal values up to 400 ng/ml during a 14 days observation period reflected the extent of the traumatic insult. From these preliminary data the conclusion could be drawn that sCD44var(v6) is neither a reliable tumor nor an inflammatory marker. Thus, further studies have to be performed to elucidate the pathological role of this adhesion molecule in tumor and inflammation.

B1-409 HUMAN MAST CELL CHYMASE AND LEUKOCYTE ELASTASE RELEASE LATENT TRANSFORMING GROWTH FACTOR-β1 FROM THE EXTRACELLULAR MATRIX OF CULTURED HUMAN EPITHELIAL AND ENDOTHELIAL CELLS, Jorma Keski-Oja[1,2], Jouko Lohi[1], Juhani Saarinen[3], Petri T. Kovanen[3] and Jussi Taipale[1], Departments of [1]Virology and [2]Dermatology and Venereology, University of Helsinki, FIN-00014 Helsinki, Finland. [3]Wihuri Research Institute, Kalliolinnantie 4, FIN-00140 Helsinki, Finland

Monolayer cultures of human epithelial and endothelial cells were used as models to study the association of latent transforming growth factor-β1 (TGF-β1) to extracellular matrices and its release and activation during matrix degradation. Human umbilical vein endothelial cells and embryonic lung fibroblasts produced relatively high levels of TGF-β1, its propeptide (β1-LAP) and latent TGF-β binding protein (LTBP), and incorporated latent TGF-β1 into their matrices as shown by immunoblotting. Amniotic epithelial cells produced lower levels of these proteins. Confluent cultures of epithelial cells were exposed to a panel of matrix degrading proteinases and glycosidases. Mast cell chymase, leukocyte elastase and plasmin efficiently released matrix bound latent TGF-β1 complexes while cathepsin G was less effective, and cathepsins B and D, bacterial collagenase, chondroitinase ABC, and heparinases I and III were inactive. Mast cell chymase and leukocyte elastase were approximately tenfold more effective in the release of matrix bound LTBP than plasmin. The latent TGF-β1 releasing proteases cleaved LTBP yielding a core fragment resembling platelet LTBP, suggesting that it has distinct functional domains. The ability of the proteinases to activate recombinant latent TGF-β1 was tested using standard biological assay and a novel Na-deoxycholate PAGE followed by immunoblotting. Na-deoxycholate solubilized M_r 25,000 TGF-β1 but did not dissociate high molecular mass latent TGF-β1 complexes, allowing separation of these forms by PAGE. Mast cell chymase and leukocyte elastase were unable to activate recombinant latent TGF-β1, suggesting that its release from matrix and activation are controlled by different proteases. The release of TGF-β from the matrix by leukocyte and mast cell enzymes and its eventual activation may contribute to the accumulation of connective tissues in a variety of inflammatory conditions.

B1-410 PLATELET DERIVED GROWTH FACTOR (PDGF) IS A CHEMOATTRACTANT FOR HUMAN MALIGNANT MESOTHELIOMA CELLS, Julius Klominek*', Dan Hauzenberger' and Karl-Gösta Sundqvist'. Dept of Lung Medicine* and Dept of Clinical Immunology', Karolinska Institute at Huddinge University Hospital, S-141 86 Huddinge, Sweden.

Malignant mesothelioma is a rare tumor that arises from serosal surfaces. Mesothelioma has a highly invasive behavior and is often seen to invade trough incisions in thoracic wall or through needle biopsy tracts. Since PDGF is involved in wound healing we investigated if malignant mesothelioma cells can migrate in response to PDGF.

Using Boyden chamber assay we found that PDGF BB but not PDGF AA is chemotactic for malignant mesothelioma cells with peak motile response at 3ng/ml. This motile response could be blocked by anti-PDGF antibodies. Using FACS analysis we found that malignant mesothelioma cells have PDGF ß receptors at the cell surface.

Preincubation of mesothelioma cells with the tyrosine kinase antagonist genistein resulted in inhibition of PDGF induced migration.

These observations indicate that malignant mesothelioma cells can migrate in response to substances secreted in the process of wound healing and may contribute to understanding why mesotheliomas invade into incisions and needle biopsy tracts.

B1-411 **Inhibition of primary tumor growth in syngeneic mice by murine urokinase receptor antagonists** Hye Yeong Min, Jennifer Stratton-Thomas, Catherine Zandonella, Charles Vitt, and Steven Rosenberg, Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608

Urokinase (uPA) has been implicated in a variety of invasive biological processes such as tumor metastasis, trophoblast implantation, inflammation, and angiogenesis. Urokinase is a serine protease which consists of three domains, an EGF-like domain (residues 1-50) comprising the receptor binding determinants, a Kringle domain (residues 51-140) and a serine protease catalytic domain (residues 180-411). uPA binds to cell surfaces via a specific receptor (uPAR) and converts plasminogen to plasmin more efficiently on the surface of cells than in solution, suggesting that cell surface uPA is of major importance in in vivo plasminogen activation. uPAR mRNA has been shown to be up-regulated in migrating endothelial cells (Pepper, et al., J. Cell Biol. 122:673 (1993)), consistent with a role for surface-bound urokinase in angiogenesis.

We have used baculovirus infected insect cells to express a murine uPA receptor antagonist consisting of the murine uPA EGF-like domain fused to the human IgG constant region ("m1-48/Ig"). This protein has a long in vivo plasma half-life and inhibits murine uPAR binding with an IC50 <1 nM. To ask whether m1-48/Ig could inhibit primary tumor growth, we injected B16 melanoma cells mixed with Matrigel and m1-48/Ig subcutaneously into syngeneic mice. Tumor volumes were measured at Days 10-21 using calipers. In mice treated with the uPAR antagonist, tumor growth was significantly inhibited in a dose-dependent manner compared to control. However, in vitro growth of B16 tumor cells was not affected by m1-48/Ig, suggesting that the urokinase receptor antagonist suppressed tumor growth by inhibiting angiogenesis. Histological studies are underway to quantify angiogenesis and uPAR expression in these tumors. This represents the first in vivo demonstration that cell surface uPA likely plays a role in tumor angiogenesis.

B1-412 A SMALL MOLECULAR WEIGHT G-PROTEIN, *rho p21*, ACTIVATES PROTEIN KINASES AND INDUCES PROTEIN TYROSINE PHOSPHORYLATION. Shuh Narumiya, Naokazu Kumagai, and Narito Morii, Department of Pharmacology, Kyoto University Faculty of Medicine, Sakyo-ku, Kyoto 606, Japan

rho p21 is a *ras p21*-related small molecular weight GTP-binding protein. This protein is specifically ADP-ribosylated by C3 exoenzyme produced by *Clostridium botulinum,* and inactivated. Studies using this enzyme and several *rho* mutants as probes have revealed that *rho p21* is activated in response to extracellular stimuli and induces activation of integrin molecules and stress fiber formation to stimulate the cell-substrate adhesion. *rho p21* is also suggested to regulate the contractile ring formation during cytokinesis. In order to elucidate the molecular mechanism of these actions, we used botulinum C3 exoenzyme and examined its effects on lysophosphatidic acid (LPA)-induced activation of protein kinases and tyrosine phosphorylation. Subconfluent cultures of Swiss 3T3 cells and rat 3Y1 cells were subjected to serum starvation and incubated with or without C3 exoenzyme for 48 h. The cells were then washed and stimulated by LPA. After incubation, the cells were lysed with the modified RIPA buffer and the lysates were used for the analyses. The immunoblot and immunoprecipitation experiments showed that LPA stimulation induced tyrosine phosphorylation of a number of cellular proteins including p43 ERK-2, p64, p72 paxillin, p88 and a group of proteins of 110-130 kDa including p125 FAK. The treatment with C3 exoenzyme induced the *in situ* ADP-ribosylation of *rho p21* in the cells and significantly suppressed the LPA-stimulated phosphorylation of ERK-2, paxillin and FAK. In order to examine protein kinases involved in this process, the renaturation kinase assay was performed in the cell lysates. This analysis revealed that LPA activated protein kinases of 43, 60, 64, 85 and 145 kDa. Among them, activation of 60/64 kinases was significantly suppressed by the prior C3 exoenzyme treatment. These results suggest that *rho p21* activates a kinase cascade and induces tyrosine phosphorylation of cellular proteins in LPA signalling. Beacuse LPA induces the integrin-mediated cell adhesion via *rho p21*, the above mentioned activation of a kinase cascade may underlie the stimulus-evoked adhesion of cells to substratum.

B1-413 INFLUENCE OF ANTIESTROGENS AND GROWTH FACTORS ON THE INVASIVENESS OF BREAST CANCER CELLS IN CULTURE, J. Thomas Pento, Talitha T. Rajah, S.M. Abbas Abidi and Gina M. Hurt, Department of Pharmacology and Toxicology, University of Oklahoma, Health Sciences Center, Oklahoma City, OK 73190

The influence of antiestrogens [tamoxifen (TAM), ICI-182,780 (ICI) and Analog II (1,1dichloro-Cis-2,3-diphenylcyclopropane)(AII)] on invasiveness and the chemotactic potential of growth factors [EGF, IGF-1 and TGF-ß] was examined in MCF-7 (ER+) and MDA-MB-231 (ER-) human breast cancer cells. In addition, cell morphology was examined using scanning electron microscopy (SEM) and type IV collagenase release was assayed. In the invasion assay, cells were treated with antiestrogen (10^{-10}-10^{-6}M) for 4 days and seeded ($3x10^5$cells/well) onto the upper surface of Matrigel coated (100 µg/sq cm) nucleopore membranes (Costar Transwells; 12 µm pore) and incubated for 24 hrs in the presence of antiestrogen. In the chemotaxis assay, cells were treated with antiestrogen (10^{-6}M) for 4 days and seeded ($3x10^5$ cells/well) onto the upper surface of collagen coated (1.5 µg/sq cm) nucleopore membranes (8 µm pore) and allowed to migrate for 6-12 hrs with growth factors (10^{-8}M) added to the lower chamber. Cells on the membrane surface were then fixed, stained with Diff Quik and invasiveness or chemotaxis determined by counting cells in 10-20 fields on the lower surface. In addition, collagenase activity in media was determined. The results indicate that AII and TAM (10^{-6}M) caused a 20 % reduction in the invasiveness of MDA cells. It was further determined that collagenase release from MDA cells, which was greater than from MCF cells, was reduced by AII and ICI (10^{-6}M) treatment. It was observed by SEM that MDA cells developed cell surface microvilli and cellular extensions "invadopodia" that reached into membrane pores. TGF-ß was found to be more active than EGF or IGF-1 in stimulating tumor cell chemotaxis. In conclusion, our results indicate that AII and TAM reduced tumor cell invasiveness and that ICI produced the greatest inhibition of collagenase release in vitro. The antiestrogens produced minor changes in growth factor-mediated tumor cell movement. (This project was supported in part by OCAST grant HR2-009 and NIH/NCI grant CA 62117.)

B1-414 MOTILITY OF HEPATOCYTES AND HEPATOCELLULAR CARCINOMA CELLS IN RESPONSE TO LIVER REGENERATIVE FACTORS IN VITRO. Donna Beer Stolz, Wendy M. Mars and George K. Michalopoulos. University of Pittsburgh School of Medicine, Department of Cellular and Molecular Pathology, Pittsburgh, PA 15261

As a result of mechanical or chemical injury to the liver causing hepatocyte loss, the liver responds by regeneration to its original mass. We have investigated the effect of the growth factors hepatocyte growth factor (HGF), epidermal growth factor (EGF), transforming growth factor-β1 (TGF-β1) and the serine protease urokinase-type plasminogen activator (uPA) on primary rat and human hepatocytes as well as two human hepatocellular carcinoma cell lines in order to examine their role in motility during the regenerative process. In human and rat primary hepatocyte cultures grown on collagen type I in serum-free medium, 50 ng/ml HGF or EGF stimulated the motility 5.5 and 4 fold respectively and DNA synthesis 2 fold at 24 hr. Although DNA synthesis was abrogated to near control levels upon concomitant addition of 1 ng/ml TGF-β1 to these cultures, the HGF-induced motility was not affected and the EGF-induced motility increased to 7 fold over controls. Therefore, simultaneous addition of TGF-β1 to either HGF or EGF-stimulated cultures shuts down the proliferative effects of the growth factors, but not the motogenic responses in primary hepatocytes. Additionally, when uPA was added to cultures stimulated with either HGF or EGF, HGF-induced motility was significantly enhanced, but EGF motility remained unaffected. HepG2 and Hep3B human hepatocellular carcinoma cell lines were also examined for their response to HGF, EGF and TGF-β1. Hep3B were hyper-motile under all assay conditions. HepG2 cells, however, displayed 3 fold increased motility with HGF and nearly 20 fold with EGF. Addition of TGF-β1 slightly enhanced both HGF and EGF-induced motility. Parallel DNA synthesis assays indicated that hepatocellular carcinomas did not respond to any cytokine. The data suggest that separate growth factors involved in liver regeneration act synergistically to modulate normal primary and transformed hepatocyte motility in vitro. These results indicate that two distinct pathways exist within hepatocytes to uniquely modulate motogenic and proliferative responses.

B1-415 HUMAN BREAST CANCER CELL LINES SUPPORT OSTEOCLAST DIFFERENTIATION IN VITRO, M. Mehrdad Tondravi, Marisol Quiroz, F. Patrick Ross and Steven L. Teitelbaum, Department of Pathology, Jewish Hospital, Washington University, St. Louis, MO 63110

Breast cancer often metastasizes to bone. These bone metastases cause osteolysis resulting in bone fractures and hypercalcemia. Although the precise mechanisms of breast cancer metastasis to bone is not understood, osteoclasts probably play a critical role. Several lines of evidence indicate that the cancer cells do not resorb bone per se, and have led us to the hypothesis that once a tumor has been established in the bone marrow environment, the cancer cells promote the differentiation of marrow-residing osteoclast precursors into mature, bone-resorbing, polykaryons. As a test of this hypothesis, we have established a cell culture system whereby breast cancer cells promote osteoclast differentiation in vitro. The osteoclast is a member of the monocyte/macrophage lineage. In mammals, differentiation of macrophage precursors into osteoclasts requires the presence of accessory cells which include stromal cells or osteoblasts. We have established that several human breast cancer cell lines (MCF-7, T47D, MDA-MB-231) can assume the role of the accessory cells in osteoclastogenesis. We are addressing whether these cancer cell lines induce osteoclast differentiation through a secratory factor by culturing the osteoclast precursors in the bottom of transwell dishes and the cancer cells on the separating filter. The results thus far, with one of the cell lines indicates that a soluble factor is not involved. We have begun to generate clonal sublines of some of our breast cancer cells and have identified clones of T47Ds which do not support osteoclastogenesis in vitro while other sublines do. Experiments are underway to test the ability of these sublines to establish tumors and cause osteolysis in vivo, in the bone marrow of nude mice.

B1-416 GROWTH FACTOR - INDUCED INVASION BY HUMAN SQUAMOUS EPITHELIAL CELLS IN AN ORGAN CULTURE MODEL, James Varani, Department of Pathology, University of Michigan, Ann Arbor, MI 48109.

Organ cultures of human skin were maintained for 8-10 days under serum-free, growth factor-free conditions. Replicate cultures from the same donor skin were maintained in culture medium supplemented with epidermal growth factor, insulin, hydrocortisone, pituitary extract and all-trans retinoic acid. Normal histological structure and biochemical function were preserved in tissue incubated under growth factor-free conditions. In contrast, a hyper-proliferative response was induced in the epidermis of growth factor-treated tissue. In this tissue, strands of epidermal cells pushed down into the space occupied by the dermis. For the most part, these epithelial strands were separated from the mesenchyme by an intact basement membrane. However, breaks in the basement membrane occurred and epithelial cells invaded the mesenchyme at these sites. A number of differences were observed between growth factor-treated tissue and control tissue. For example, $\alpha 6$ and $\beta 4$ integrins were expressed at the dermo-epidermal junction in control tissue but this staining pattern was replaced by diffuse staining throughout the basal epidermal layer in the growth factor-treated tissue. In contrast, there was no significant difference in the $\beta 1$ integrin staining pattern. Differences in extracellular matrix synthesis were also seen. Fibronectin and laminin synthesis were elevated in tissue maintained in the presence of growth factors, although there was no difference in overall protein synthesis. Finally, serine proteinase activity and metallo-proteinase activity were increased in culture fluids obtained from growth factor-treated tissue. The excess proteinase activity may be important because both serine proteinase inhibitors and an inhibitor of metalloproteinases suppressed invasive activity (assessed histologically) and reversed some of the abnormal characteristics associated with growth factor treatment.

B1-417 SYNTHETIC PEPTIDES ENCOMPASSING RESIDUES 12-32 OF UROKINASE PLASMINOGEN ACTIVATOR (uPA) POSSESS POTENT ANTI-INVASIVE, ANTI-PROLIFERATIVE AND ANTI-ANGIOGENIC PROPERTIES, Brian Walker*., William. Allen**., Andrew McDowell*; John Nelson* & David Wilson**. *Division of Biochemistry, School of Biology & Biochemistry and **School of Biomedical Science/Anatomy, Queen's University, 97 Lisburn Road, Belfast BT9 7BL, N. Ireland, U.K.

We have examined the ability of synthetic peptides derived from the amino terminal region of uPA, specifically residues 12-32 which have previously been demonstrated to displace ^{125}I-labelled uPA from its cognate receptor[1], to block the plasminogen-dependent invasion of human breast cancer cells T47D through re-constituted basement membranes (Matrigel-coated inserts) and to inhibit endothelial cell migration. We have found that in addition to inhibiting the invasion of the breast tumour cells at concentrations as low as 0.1 μM, the peptides also exhibit potent anti-proliferative activity against the cell line, block capillary endothelial cell migration (as measured by the phago-kinetic track assay) and are potent inhibitors of angiogenesis in the *in vivo* chick vitelline membrane (VIM) assay. Biotinylated versions of the peptides have also been tested and have been found to be equipotent with their un-biotinylated counterparts.

We believe that these peptides may represent novel lead compounds for the treatment of breast cancer via inhibition of tumour invasion and tumour-induced angiogenesis.

[1]Appella, G., Robinson, E. A., Ullrich, S. J., Stoppelli, M. P., Corti, A., Cassani, G. & Blasi, F. (1987) *J. Biol. Chem.* **262**, 4437-4440.

B1-418 **EXPRESSION AND FUNCTION OF AUTOCRINE MOTILITY FACTOR RECEPTOR IN HUMAN CHORIOCARCINOMA CELLS.** F.D. Yelian, A. Raz#, A.L. Liu, J. Todt, J. Lei, F. Qureshi§, S.M. Jacques§. Department of Obstetrics and Gynecology, #Michigan Cancer Foundation, and §Department of Pathology, Wayne State University School of Medicine, Detroit, MI 48201

Choriocarcinoma is a highly malignant trophoblastic neoplasm that frequently metastasizes. Previous studies have shown that tumor cell invasion and metastasis are dependent on cell motility at the advancing edge of tumor protrusions. It has been suggested that B16-F1 melanoma cells express autocrine motility factor (AMF) receptor, a 78 kDa cell surface glycoprotein (gp78), which is correlated to an increased metastatic ability *in vivo* and motility *in vitro*. In our present study, a monoclonal antibody (3F3A mAb) directed against gp78 was used to study AMF receptor expression, localization and possible function in choriocarcinoma cells. Using indirect immunofluorescence staining we have found that gp78 was highly expressed in the JEG-3 choriocarcinoma cells. The staining of gp78 was mostly localized in cell surface showing receptor clustering. There were also some punctate staining and diffuse staining in some cells. However, the staining on 3A-subE cells, a term placenta trophoblast cell line, was much less intense and no receptor clustering was observed. Two individual formalin-fixed and paraffin-embedded choriocarcinoma tissue sections were also stained with 3F3A mAb. The expression of gp78 was strongly localized at the advancing edges of the choriocarcinoma masses. However, adjacent normal villous trophoblast cells and necrotic tumor masses were staining negative, suggesting the positive correlation between gp78 expression and invasive potential. Using a phagokinetic track motility assay, we have found that gp78 positive JEG-3 cells had significantly higher motility than 3A-subE cells. Furthermore, JEG-3 cell motility was stimulated by addition of 3F3A mAb in a serum-free culture medium, suggesting that the binding of antibody to the receptor may activate cell locomotion. In contrast, the effect of this mAb on 3A-subE cells was not significant. In conclusion, human choriocarcinoma cells highly express functional AMF receptor, which may play a critical role in tumor cell motility and act as a biological marker for metastatic potential.

B1-419 **ELEVATION OF PRO-CATHEPSIN D IN THE PLASMA OF BREAST CANCER PATIENTS MEASURED BY ELISA.** James R. Zabrecky, David E. Jarosz, Peter J. Hamer and Donald Y. Tenney, Oncogene Science, Inc. Cambridge, MA 02142.

Changes in the expression and processing of cathepsin D (CD) have been shown to be associated with cancer invasion and metastasis. However, the value of CD as a prognostic marker remains controversial. Most studies have used immunological methods to measure the mature form of CD, although it is the precursor (pro-CD) that appears to be abnormally secreted by breast cancer cells. A sandwich-type ELISA has been developed that is specific for pro-CD. The assay employs a monoclonal antibody to mature CD as the capture reagent and a rabbit polyclonal to the pro fragment as the detector. The assay is specific for pro-CD and capable of quantitating this antigen in biological samples. Pro-CD levels were measured in plasma samples from 76 breast cancer patients and compared with 36 plasmas from normal control individuals. The breast cancer plasmas showed elevated levels of pro-CD that appeared to be independent of stage. Immunoblots of the plasma samples using a CD monoclonal antibody revealed a band at the appropriate size for pro-CD that corresponded in intensity with the ELISA results. These results indicate that measurement of pro-CD in plasma may be a useful prognostic indicator for breast cancer.

Late Abstracts

HIV-1 TAT PROTEIN AND BASIC FIBROBLAST GROWTH FACTOR (bFGF) SYNERGIES IN INCREASING COLLAGENASE IV RNA EXPRESSION IN NORMAL HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVEC), Sandra Colombini, Rita Gendelman, Robert C. Gallo and Barbara Ensoli, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4255

During neoangiogenesis and tumor invasion pericellular proteolysis is essential for the detachment of endothelial cells and their migration into adjacent tissue. Among the enzymes responsible for the degradation of the basement membrane, collagenase IV plays a major role (Liotta *et al.*, ***Cell*** 64:327-336, 1991). Several reports have indicated a perturbation of the extracellular matrix proteins/ metalloproteinases system in HIV-1-infected cells and it has been suggested that this effect may promote the dissemination of HIV-1 to a variety of tissues and facilitate the development and progression of AIDS-associated malignancies. Kaposi's sarcoma (KS), an angioproliferative disease, occurs at high frequency and in a clinically aggressive form in AIDS patients. Cultured spindle cells derived from KS lesions of AIDS patients produce and respond to bFGF (Ensoli *et al.*, ***Science*** 243:223-226, 1989). A comparable behavior, in response to the same stimuli is observed in normal endothelial cells considered to be the progenitors of AIDS-KS cells. Similarly, both cell types exhibit increased rate of growth and invasion through the extracellular matrix in response to HIV-1 Tat protein (Barillari *et al.*, ***J. Immunol.*** 149:3727-3734, 1992; Albini *et al.*, ***Proc. Natl. Acad. Sci. USA***, in press). We examined the role of bFGF and Tat on the expression of collagenase IV in HUVEC. We observed that both bFGF and Tat at low concentrations are able to induce a 2-fold increase of collagenase IV mRNA expression and when combined they synergize inducing 7.6-fold increase (Ensoli *et al.*, ***Nature*** 371:674-680, 1994). Since both Tat and bFGF are present in AIDS-KS lesions, they synergize in inducing KS-like lesions in mice (Ensoli *et al.*, ***Nature*** 371:674-680, 1994) and they increase endothelial cell invasion due to collagenase IV activation, they may cooperate in promoting KS lesion formation and progression in HIV-1-infected individuals.

MOTILITY FACTORS IDENTIFIED IN SUPERNATANTS OF MAMMARY CARCINOMA CELLS.

Ulrich Scherdin, Hakan Kopdag, Thomas Rau and Fritz Hölzel, Physiologisch-Chemisches Institut, Abt. Molekularbiologie, UKE, 20251 Hamburg, Germany.

Objective: Cellular motility plays an important role in embryogenesis, wound healing and metastasizing of tumor cells. A number of normal and neoplastic cell types produce motility factors, four of which (SF, AMF, MSF, ATX) were characterized previously. Additional factors possibly involved in the induction of tumor cell invasion and metastasizing may be obtained from supernatants of malignant tumor cell cultures.

Materials and methods: Conditioned medium of malignant cell cultures was mixed with growth medium of different indicator cell lines (NRK, MDCK, MCF-7, MFM-223). Dispersion of cellular colonies (scattering) was assayed microscopically after 2-3 days of cultivation.

Results: Supernatants derived from mammary carcinoma cell cultures of rat (HH-16 cl.4) and human origin (MDA-MB 231, SKBR-7) contain scattering activity stimulating the migration of at least one of the indicator cell lines. None of the supernatants induced scattering of the same set of indicator cells, indicating that the supernatants do not contain identical motility factors. Scattering activity (CL4-SA) produced by HH-16 cl.4 rat mammary carcinoma cells was analysed in detail. CL4-SA is a heat-labile and trypsine-sensitive protein with a molecular weight between 50 and 100 KD that does not bind to concavalin A. It is neither inhibited by heparin nor affected by the presence of TGF-β1. In addition to scattering, CL4-SA stimulates migration of NRK and MFM-223 indicator cells across 8 µm microporous membranes in Boyden chamber chemotaxis assays.

Conclusion: Supernatants from three mammary carcinoma cell lines contain scatter factors one of which (CL4-SA) may not be identical whith other motility factors described recently.

Cloning of the TIMP-1 Genomic Regulatory-Promoter from PC-3 Human Prostate Tumor Cells.

Mark E. Stearns and Min Wang. Department of Pathology, Medical College of Pennsylvania, 3300 Henry Avenue, Philadelphia, PA 19129

We have recently cloned the human genomic TIMP-1 gene (4 Kb) including the coding and regulatory-promoter regions from highly non-invasive PC-3 human prostate sublines (see Wang and Stearns, 1991, Differentiation, 48: 115). We have characterized the regulatory region by restriction endonuclease mapping and identification of potential regulatory sequences based on gel retardation, DNA footprinting, and pCAT transfection assays. A 18 bp cis-acting regulatory element (ATGCCACGATGACTCATCA) has been identified 748 bp upstream of the 5' ATG start site which strongly upregulates pCAT activity in transfected PC-3 ML clones in response to IL-10 (10- 20 ng/ml-2 hr) and IL-6. Currently we are subcloning regions of the 5' promoter region for sequencing, in vitro transcription and in vivo transfection analysis. These experiments should demonstrate if independent regulatory sequences (and factors) control TIMP-1 synthesis.

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EFFECTS OF SYSTEMIC ADMINISTRATION OF A CATHEPSIN B INHIBITOR ON METASTATIC GROWTH OF COLON CANCER CELLS IN THE RAT LIVER, Cornelis J.F. Van Noorden*, Trudy G.N. Jonges**, and Robert E. Smith***, *Laboratory of Cell Biology and Histology, Academic Medical Center, University of Amsterdam, The Netherlands; **Department of Pathology, Academic Hospital Utrecht, The Netherlands; ***Prototek, Dublin, CA 94568

A rat model of colon cancer metastasis in the liver was used to investigate the role of 2 proteinases, cathepsin B and urokinase-type plasminogen activator (uPA), in this process. Colon adenocarcinoma cells were obtained by treatment of rats with 1,2-dimethylhydrazine and were injected in a single cell suspension (10^6 cells) in the vena porta of male rats. After 1 week a mean of 30 metastases per unit volume liver were found in the 3 large lobes of the livers, whereas a mean of 70 metastases were found in the small caudate lobe. The protein and activity of the proteinases were localized with immunocytochemical and enzyme histochemical means in the cancer cells before injection and in sections of the livers 1 week after injection. Cathepsin B protein and activity was present in a granular form inside the cancer cells and on the plasma membrane as demonstrated by confocal scanning laser microscopy. One week after injection, the cancer cells in the livers showed the protein but not its activity. uPA activity could not be found in the cancer cells either before or after injection and only in a few stromal cells around metastases and in the connective tissue of portal tracts. This localization pattern was similar in control rats and in rats bearing metastases. Daily i.v. treatment of rats with Z-Phe-AlaFMK, a selective inhibitor of cathepsin B, altered the growth of metastases significantly. In the large lobes, the number of metastases was decreased by 75% to a mean of 8 metastases, but in the caudate lobe the number was increased 2-3 fold to 180 metastases. It is concluded that cathepsin B but not uPA plays a significant role in invasion and metastasis in this rat model and that inhibition of cathepsin B modulates these processes.

APOLIPOPROTEIN E: A NOVEL LIPID/HEPARIN-BINDING PROTEIN INHIBITS DEVELOPMENT OF KAPOSI'S SARCOMA-LIKE LESIONS IN NUDE MICE. Vogel T, PB Browning, B Ensoli, Y Watanabe, DD Roberts, J Bryant, RC Gallo, and A Panet. BioTechnology General, Ltd. Rehovot, Israel (TV,AP); NCI (DDR, BE, RCG), and NIDR (JB), NIH, Bethesda, MD 20892; Vanderbilt University, Nashville, TN 37232 (PJB). Apolipoprotein E3 (apoE) is ubiquitously expressed, and binds with high affinity to lipids and to heparin and its derivatives. We have used a recombinant human apoE and demonstrated that this protein inhibits vascular endothelial cell growth, adhesion and migration, in vitro, and postulated that apoE may be anti-angiogenic (Vogel et al (1994), J Cell Bioch 54:299). We have now tested the effect of apoE in an in vitro and in vivo model systems for AIDS-associated Kaposi's sarcoma (KS). Our results revealed that apoE blocked the proliferation and motility of AIDS-KS-derived spindle cells in response to activated lymphocyte conditioned media, or to oncostatin M. Moreover, this protein inhibited the formation of neoangiogenic lesions induced in nude mice by AIDS-KS-derived spindle cells. ApoE also completely blocked the induction of vascular hyper-permeability and edema induced by AIDS-KS cells in nude mice. Inhibition of cell proliferation by apoE was cytostatic, blocking the transition of the cells from Go to S phase. These findings represent a novel and potentially less toxic therapeutic approach for treatment of AIDS-associatd KS.