337
INVASIVENESS OF ENDOTHELIAL CELLS: ROLE IN TUMOR ASSOCIATED ANGIOGENESIS

A. Albini, R. Benelli, D. Giunciuglio, L. Masiello, G. Fontanini[1], D. Noonan
Ist. Naz. Ric. Cancro (IST), Genova
[1]Ist. di Anat. Pat., Univ. Pisa, Italy

Neoangiogenesis, the formation of new capillary vessels, is essential for tumor growth and the formation of metastases. This process requires that the activated endothelial cells invade their own basement membrane. We have used the reconstituted basement membrane Matrigel model (Albini et al., Cancer Res. 47: 3239, 1987) to study endothelial cell (EC) invasion *in vitro*. Supernatants (CM) of cells from Kaposi's sarcoma (KS), a highly angiogenic neoplasm strongly induce EC invasiveness. The activity of gelatinase-A, an enzyme closely linked with the invasive phenotype of metastatic cells, is enhanced in endothelial cells treated with KS-CM. Exogenous TIMP-2 is capable of suppressing endothelial cell invasion stimulated by KS products. Inhibition is also induced by a synthetic peptide from the propeptide region of gelatinase A, containing a conserved unpaired cysteine residue. bFGF (a product of KS cells) as well as KS-CM are strongly angiogenic in the matrigel sponge model *in vivo*. TIMP-2 can suppress bFGF induced neovascularization of the sponges and inhibits KS products associated angiogenesis. Histological examination shows that no endothelial cells were present in the TIMP-2 treated bFGF sponges, confirming that endothelial cell invasiveness is necessary for tumor angiogenesis. We are now attempting to use N-acetyl-cysteine, a chemopreventive agent which also interferes with metalloprotease activity, as potential antiangiogenic drug *(AIDS & CNR grants)*

338
HLA ANTIGENS IN HUMAN TUMOURS

F. Garrido
Department Análisis Clínicos e Inmunologia, Hospital Virgen Nieves, Universidad de Granada 18014 Granada, Spain

HLA expression is frequently altered in tumours compared to the tissue from which they originate. Given the central role of MHC products in the restriction of T-cell recognition, regulation of tumour HLA expression might be a strategy for the evasion of immune surveillance by the malignant cells. Three major types of altered HLA tumour phenotypes have been defined: HLA total losses, HLA-A or B locus specific losses and HLA allelic losses. Each of these altered phenotypes correspond to a particular mechanism responsible for the HLA down regulation. Data will be presented indicating the frequency of HLA losses in different tumour types as well as the timing of this phenomenon in the multistep process of tumour development. The clinical implications of these findings will be discussed.

339
STRUCTURAL AND FUNCTIONAL ANALYSES OF THE INVASION AND METASTASES INDUCING TIAM1 GENE PRODUCT

G.G.M. Habets, F. Michiels, R.A. van der Kammen, J. Stam, F. van Leeuwen, J.G. Collard
The Netherlands Cancer Institute, Division of Cell Biology, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

We recently isolated the Tiam1 gene from retrovirally mutagenized T-lymphoma cells (Cell 77, 537–549, 1994). The encoded Tiam1 protein harbors a Dbl homology (DH) domain, which is also present in activators (GDS) of Rho-like GTPases. These GTPases have been implicated in the regulation of the actin cytoskeleton in response to distinct extracellular stimuli and control the morphology, adhesion and motility of cells. Tiam1 indeed encodes an activator (GDS) of the Rho-like Rac protein (Nature, 1995 in press). Both, constitutive active V12Rac1 and Tiam1 induce invasiveness in T-lymphoma cells, suggesting that the Tiam1-induced invasion is caused by activation of Rac. Tiam1 induces a similar morphologically transformed phenotype in NIH3T3 fibroblasts as V12Rac1, including the formation of membrane ruffles. The Tiam1- and V12Rac1-transformed fibroblasts also produce tumors in nude mice. These studies implicate the Tiam1-Rac pathway in the process of tumor formation, invasion and metastasis.

340
ROLE OF ADHESION RECEPTORS IN TUMOUR SPREAD

I.R. Hart, Z. Seller, A. Saini, J.F. Marshall
Richard Dimbleby/ICRF Department of Cancer Research, St. Thomas's Hospital, London SE1 7EH, U.K.

Since tumour spread involves numerous cell to cell and cell to matrix interactions it is expected that alterations in expression of cell adhesion receptors will play a significant role in modulating metastasis. Our interests have focused primarily upon the adhesion receptors involved in regulating the spread of malignant melanoma. Immunocytochemistry of clinical specimens has shown that the $\alpha_4\beta_1$ integrin is expressed in melanoma but that this expression is restricted to metastases. We have identified two α_4 deficient melanoma cell lines, HMB2 and VUP, which have had $\alpha_4\beta_1$ expression reconstituted by infection with retroviruses carrying α_4 cDNA. Such reconstituted VUP and HMB2 lines are able to bind to VCAM-1, an inducible endothelial cell adhesion molecule expressed on the surface of activated endothelium, and that such binding is inhibited with an α_4 blocking antibody HP21. Binding of anti α_4 antibody to the α_4 expressing melanoma cell lines results in the induction of second messenger molecules, such as a calcium spike, and changes not only in adhesive but also in a migratory capacity. Thus, migration on VCAM-1 by human melanoma cells is $\alpha_4\beta_1$ dependent suggesting that adhesion molecules expressed during tumor progression may play substantial roles in different steps of the metastatic process.

341
PROTEASES IN BREAST CANCER

F. Spyratos, J. Oglobine
Centre René Huguenin, 92211, Saint-Cloud, France

Cancer invasion is dependent on a combined action of several proteolytic enzymes, such as collagenases, other metalloproteases, and serine proteases. Cathepsin-D was the first example of a protease whose immunoassay in the cytosol of breast tumors was available and could be readily standardized with quality control. Most studies indicated a worse prognosis in high Cathepsin-D tumors. Urokinase-type plasminogen activator (UPA), urokinase receptor (UPAR), and 2 plasminogen activator inhibitors, PAI1 and PAI-2, are all involved in regulation of plasmine generation. UPA and PAI1 levels in breast cancer tissue are independant and significant prognostic markers and high levels of each of the parameters are associated with poor prognostic. In tumors with high UPA or PAI1 content, a high level of PAI-2 appeared to be an independant marker of favorable prognostic. However, components of proteolytic enzyme systems are often expressed in non malignant stromal cells in invasive areas of cancer tissue. The cellular expression pattern of these molecules appears to be characteristic for each type of cancer and is not yet fully clarified. The measurements of proteases could be helpful for classifying breast tumors for understanding processes of breast tumor invasion and metastasis, and for the development of future treatment strategies based on interference in the proteolytic cascade which lead to tumor dissemination.

342
MULTIFUNCTIONAL GROWTH FACTORS DURING TUMOR PROGRESSION

J. P. Thiery
CNRS, and the Curie Institute, Paris, France

We have made the hypothesis that the progression of in situ carcinoma to invasive and metastatic stages implicate the deregulation of mechanisms ensuring the maintainance of stable epithelia. In our laboratory, we have developed a suitable model system to study the molecular basis of epithelial-mesenchymal interconversions. We have used an epithelial tumor cell line (NBT-II) which can convert into motile fibroblastic-like cells upon exposure to several growth factors including acidic FGF (FGF-1), EGF and Scatter Factor/Hepatocyte Growth Factor. We have analyzed the specific mechanisms whereby FGF-1 can act alternately as a mitogen or a scatter factor. I will describe early events in the transduction pathways activated by FGF-1 signalling through a tyrosine kinase surface receptor. The scatter activity which is observed only in subconfluent cultures is characterized by an early activation of c-src. NBT-II epithelial cells can also undergo an epithelial-mesenchymal transition upon exposure to collagens. The a2b1 integrin turned out to be the collagen receptor. Type I collagen and FGF-1 act synergistically to promote scatter, locomotion and invasive properties of the NBT-II

carcinoma line, most likely through an up-regulation of the a2b1 integrin. Transfection of an expression vector coding for FGF-1 into epithelial cells converted them into fibroblastic-like cells, endowed with motile, invasive and highly tumorigenic and metastatic properties. *In vivo*, the FGF-1 transfected NBT-II cells allowed untransfected cells to grow and metastasize as rapidly, thus unraveling the role of certain subsets of highly malignant cells in the progression of a heterogenous tumor cell population. These studies emphasize the multifunctional properties of some growth factors as morphogens, mitogens and motogens. These growth factors play a major role in the regulation of epithelial cell plasticity.