S6. PLASMINOGEN ACTIVATION AND CANCER: BASIC MECHANISMS AND PERSPECTIVES

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During cancer invasion, breakdown of the extracellular matrix is accomplished by the concerted action of several proteases, including the serine protease plasmin and a number of matrix metalloproteases (MMPs). The activity of each of these proteases is regulated by an array of activators, inhibitors and cellular receptors. Thus, the generation of plasmin involves the pro-enzyme plasminogen, the urokinase type plasminogen activator uPA and its pro-enzyme pro-uPA, the uPA inhibitor PAI-1, the cell surface uPA receptor uPAR, and the plasmin inhibitor α_2-antiplasmin. The plasminogen activation system appears to be active in virtually all types of cancer, while various MMPs appear to be active more selectively in different types of cancer.

Generation of extracellular proteolysis in cancer involves a complex interplay between cancer cells and non-malignant stromal cells which has far-reaching consequences for our understanding of both carcinogenesis and establishment of metastases. For some types of cancer, the cellular interplay mimics that observed in the tissue of origin during non-neoplastic tissue remodelling processes. We propose that cancer invasion is considered as uncontrolled tissue remodelling.

Inhibition of extracellular proteases is an attractive approach to cancer therapy. Because the proteases have many functions in the normal organism, efficient inhibition will have toxic side effects. In cancer invasion, like in normal tissue remodelling processes, there appears to be a functional overlap between different extracellular proteases. This redundancy means that combinations of protease inhibitors must be used. Such combination therapy, however, is also likely to increase toxicity. Therefore for each type of cancer, a combination of protease inhibitors that is optimised with respect to both maximal therapeutic effect and minimal toxic side effects needs to be identified.

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S7. THE UROKINASE RECEPTOR, CELL MIGRATION, PROLIFERATION AND CANCER

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uPAR is a GPI-anchored protein that signals by interacting with extracellular matrix proteins, transmembrane tyrosine kinase receptors, integrins and G-protein coupled receptors. uPAR regulates adhesion by either direct RGD-independent binding to vitronectin (VN), or by forming complexes with integrins. In this case uPAR appears to have higher affinity for alpha5 > alpha3 > alpham. uPAR can both activate and inactivate integrins and induce signaling via integrins or via other receptors. A seven trans-membrane G-protein coupled receptor, FPRL1, directly interacts with a uPA-cleaved form of uPAR (which cannot bind integrins) and transmits a chemokine-like signal inducing chemotaxis. In addition, uPAR can also interact with the EGF-receptor and induce either cell proliferation (via the ERK pathway) or cell migration. The choice between these two effects of uPAR may be dependent on different conformations of uPAR and hence on different types of interactions in different cells.

An important novel feature of uPAR is its involvement in the mobilization of hematopoietic stem cells. Indeed, uPAR Ko mice do not mobilize HSC, but this property can be rescued by administering a soluble form of uPAR or one of its fragments, D2D3. We have set up the tools and methodology for measuring the formation and the level of circulating D2D3 in human biological fluids since HSC mobilization is an important aspect of leukemia and lymphoma therapy. Indeed, the availability of a specific D2D3 assay allows the determination of the level of this fragments in all types of cancer and hence the correlation with the stage of the tumor.

Overexpression is one of the mechanisms transforming protooncogenes into oncogenes. In human tumors uPAR is almost invariably overexpressed. UPAR overexpression controls cell proliferation by constitutively activating integrins and growth factor receptor pathways. However, recent data on cells carrying no oncogenes mutation (embryonic mouse fibroblasts, MEF) have shown that the absence of uPAR causes an increase in cell proliferation rate and delays culture-induced senescence. On the other hand, overexpression of uPAR in MEFs causes senescence. In this type of activity, key regulatory proteins of the p53/Rb pathways are involved.

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S8. INVOLVEMENT OF p38-SAPK AND ENDOPLASMIC RETICULUM-STRESS SIGNALING PATHWAYS IN THE INDUCTION OF CANCER DORMANCY AND DRUG RESISTANCE

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Most patients with inoperable primary cancer, with or without overt metastases, or patients with undetected disseminated disease undergoing surgery for their primary cancer, are not cured by adjuvant chemotherapy. It is thought that residual tumor cells in patients with disseminated disease might exit proliferation and activate a survival and G_0/G_1 arrest that allows them to become dormant. The mechanisms that trigger and maintain dormancy are not well understood and the assumption that the lack of proliferation of dormant cells is the only reason for their resistance to chemotherapy remains to be proven. The elucidation of the molecular basis of dormancy is of fundamental interest. We have shown that a rapidly tumorigenic and spontaneously metastasizing human carcinoma (T-HEp3) that is

passaged for a prolonged period of time in culture, undergoes a non-clonal change reflected in lower ERK activity, increased p38 activity and a dormant/quiescent phenotype in vivo (D-HEp3). When inoculated in vivo ~80% of D-HEp3 cells rapidly arrest in G_0/G_1 by day 6 after inoculation and remain dormant for several months. We also found that activated p38 establishes a negative feedback loop to ERK and that blocking of p38 by genetic or pharmacological inhibitors restores ERK activation and interrupts tumor dormancy in vivo. These studies implicated high p38 activity in the induction of dormancy in vivo. While p38 is known to induce growth arrest and/or apoptosis, there is also evidence indicating that in some instances, p38 signaling can promote cell survival. However, knowledge of the proximal targets of p38 that underlie the p38-dependent dormancy program, in particular the balance between cell proliferation and cell death, have not been identified. We now show that p38 regulates the activation of the endoplasmic reticulum (ER)-stress activated kinase PERK and expression of the ER chaperone BiP/Grp78. Regulation by p38 of these pathways allows dormant tumor cells to not only become dormant but also resist drug-toxicity. Increased activation of the eIF2α kinase PERK, results in upregulation of ATF4 and activation of GADD153 promoter. RNAi and dominant negative expression studies revealed that both BiP and PERK promote survival and drug-resistance of dormant cells and that BiP upregulation prevents Bax activation. Further, genetic experiments showed that activation of the PERK-eIF2α pathway is important for the maintenance of dormancy. We propose that stress-dependent activation of p38 that results in BiP upregulation and PERK activation may represent a novel growth arrest and survival mechanism that induces dormancy and protects dormant tumor cells from stress-insults such as chemotherapy.

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S9. ZNF306, A NOVEL ZINC FINGER TRANSCRIPTION FACTOR, DRIVES COLON CANCER PROGRESSION – AN ALTERNATE GENETIC PATHWAY IN TUMOR PROGRESSION?

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Colorectal cancer is the second leading cause of cancer deaths in western countries. Although inactivation of the *APC* and *p53* tumor suppressor genes coupled with Kirsten-*Ras* oncogene activation contribute to colon carcinogenesis, simultaneous mutation of these three genes is rare suggesting alternate genetic pathways leading to colon tumorigenesis/progression. To identify novel genes that contribute to colon cancer development/progression we "data-mined" genes aberrantly expressed in colorectal cancer using SAGE and UniGene Cluster Expression analysis and identified, a novel Scan domain-containing zinc finger protein (ZNF306) whose expression is elevated in colon cancer. RT-PCR analysis of resected colon cancers showed elevated ZNF306 mRNA levels in two thirds of tumors compared with paired adjacent non-malignant tissue. Stable expression of the cloned ZNF 306 cDNA in HCT 116 colon cancer cells yielded enhanced soft agar colony formation, anoikis resistance and resistance to 5-flurouracil when compared with cells bearing the empty vector. More importantly, orthotopic implantation of ZNF306-overexpressing HCT 116 cells yielded large tumors in 100% of the mice compared with vector only-expressing cells which produced smaller tumors with a lower penetrance (20% of mice). Conversely, transduction of two independent colon cancer cell lines with a-ZNF306 siRNA reduced mRNA levels, diminished colony size and attenuated cell proliferation. Further, in vivo delivery of neutral liposomal-encapsulated siRNA targeting ZNF306 reduced orthotopic growth of HCT116-ZNF306 cells. Flag-tagged expressed ZNF306 was nuclear-localized and since zinc finger-containing proteins recognize DNA site-specific sequences, we hypothesized that ZNF306 is a transcription factor. Cyclic amplification and selection of targets (CAST-ing) using a random oligonucleotide library identified the KRKGGGG nucleotide sequence as a putative DNA binding site. Expression profiling studies revealed several candidate downstream targets of ZNF306 including VEGF and integrin β4, implicated in angiogenesis and Ras/PI3-kinase signaling, respectively. Over-expression of these two putative targets was confirmed by RT-PCR. Additionally, increased CD31 (endothelial cells) immunoreactivity in the ZNF306-over-expressing orthotopic tumors indicated augmented angiogenesis. Both genes contained ZNF306 binding sites in their regulatory sequences and chromatin immunoprecipitation assays and EMSA, using an anti-ZNF306 antibody we generated, demonstrated binding of the ZNF306 protein to its recognition sequence (identified by CAST-ing) in the VEGF promoter indicating this gene to be a direct target of ZNF306. Immunohistochemistry employing the anti-ZNF306 antibody showed increased ZNF306 protein in colon cancer tissues compared with adjacent non-malignant mucosa. In conclusion, we have discovered a novel zinc finger protein, ZNF306 that contributes to colon cancer progression in part by elevating VEGF and integrin β4 expression. We propose that this gene product represents a key protein in an alternate genetic pathway leading to colon cancer progression.

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S10. SPECIFIC TRANSCRIPTIONAL REGULATORS OF THE u-PAR GENE – IN VIVO AND CLINICAL RELEVANCE, AND FIRST SUGGESTIONS FOR MOLECULAR TUMOR STAGING

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The urokinase-receptor (u-PAR) promotes the invasive and metastatic phenotype and has been shown to be associated with early relapse and poor prognosis in enumerous types of cancers. From our and other studies we know that high u-PAR gene expression in carcinoma cells is largely due to the transcriptional regulation of the gene. We have characterized two *cis*-elements (−152/−135, bound with an AP-2-like protein, Sp1, and Sp3; −190/−171, bound with AP-1-transcription factors) of the u-PAR promoter which are decisive for diverse means of u-PAR-gene expression in highly invasive colon cancer cells, among them being constitutive,