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,

PMA-induced, K-ras- and Src-induced gene expression. New data will be presented in this lecture showing that the newly characterized tumor suppressor Pdcd4 suppresses u-PAR gene expression, this again in part being mediated by Sp3 bound to the −152/−135 motif.

Furthermore, the lecture will focus on the differential binding of transcription factors to both u-PAR promoter elements in vivo, having been investigated in a large series of resected tumor and normal tissue of colorectal and gastric cancer patients. We will demonstrate that, depending on the transcription factor and *cis*-element, patient subgroups of different size can be selected in which transactivation via these promoter elements might be tumor-tissue-specific, suggesting subgroups for tumor-selective targeting. Also, the lecture will outline that different u-PAR-promoter motifs may be of different tumor-specificity in vivo. We will also suggest patient subgroups in which a synergistic regulation of u-PAR gene expression in resected tissues via both promoter elements can be postulated. Finally, first data on a clinical-prognostic relevance of differential transcription factor binding to specific u-PAR-promoter motifs will be shown, suggesting the binding of, for example, Sp1, and transcription factor combinations out of Sp1/AP-2 and AP-1-binding as new and independent predictors of disease-specific survival. A first molecularly extended staging model will be presented from these data. Potential conclusions for a more target-oriented patient selection and therapy out of transcriptional and oncogeneic regulators of the uPA-R gene will be discussed.

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S11. uPA AND PAI-1: CLINICALLY AND TECHNICALLY VALIDATED PROGNOSTIC MARKERS IN BREAST CANCER

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For optimum management of patients with cancer, accurate prognostic factors are required. The primary determinant of outcome in patients with malignancy is cancer progression, especially the formation of distant metastases. Based on data from model systems, urokinase plasminogen activator (uPA) is one of the critical mediators of cancer progression. uPA appears to mediate progression via multiple mechanisms including remodelling of the extracellular matrix, enhancing cell proliferation and migration and modulating cell adhesion. PAI-1, although originally identified as an inhibitor of uPA, is also causally involved in cancer progression. Consistent with their roles in cancer progression, multiple independent studies have shown that elevated levels of uPA and PAI-1 predict poor prognosis in patients in breast cancer. The prognostic impact of uPA and PAI-1 is potent (e.g., RR > 2.0), independent of standard prognostic factors and found in both lymph node-negative and lymph node-positive disease. Importantly, the prognostic impact of uPA/PAI-1 has been validated in both a randomized prospective trial and a pooled analysis, i.e., in 2 level I evidence studies. In addition to clinical validation, specific ELISA for uPA and PAI-1 have undergone technical validation including validation in an external quality assurance program. uPA and PAI-1 are thus now ready for clinical application, especially in the identification of newly diagnosed breast cancer patients that may be able to avoid having to receive adjuvant chemotherapy.

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S12. EXPRESSION OF MARKERS OF INVASION AND PROGRESSION – COMPARING MOLECULAR DETERMINANTS WITH PHENOTYPES

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Cancer cells usually have a distinct morphological and genetic profile that allows to determine between reactive, premalignant and malignant lesions. In some tumor entities, however, morphological and expression profiles do not necessarily reflect the true nature of the putative lesion. Molecular biological advances could further clarify the biological potential in some tumor entities, in others however, morphological and topographical criteria are still crucial, since no evident typical molecular profiles could be determined so far.

Prostate cancer is a prominent example of cancer where exocrine and neuroendocrine (NE) tumor cells can occur within the same tumor. The role of these NE cells is still under debate, even the question of its neoplastic nature and its biological significance. Since in these NE cells no proliferation could be demonstrated so far, NE tumor cells in prostate cancer are regarded as post-mitotic and their significance has been regarded as 'low'. The interesting question is, therefore, whether a post-mitotic cancer cell still deserves the attribute "cancer cell" and what are its biological functions in the cancerous orchestration. Although molecular and clinical data seem to give evidence that NE tumor cells are the result of a transdifferentiation process and possess a prognostic significance, their final role in vivo is not yet completely understood.

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S13. MOLECULAR MECHANISMS OF MATRIX METALLOPROTEINASE (MT-MMP) INDUCTION OF CANCER CELL MIGRATION AND METASTASIS

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Matrix metalloproteinases (MMPs) are important in cancer dissemination by virtue of degradation of extracellular matrix, as well as diverse effects on cell growth, apoptosis, migration, and angiogenesis. Negative results from clinical drug trials of MMP inhibitors in advanced cancers has refocused attention on the role of MMPs in early cancer development. Experimental and clinical evidence suggests that membrane type 1-matrix metalloproteinase (MT1-MMP) may serve as a master regulator of cancer progression. The mechanism underlying this process