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

remains to be defined. Epithelial-to-mesenchymal transition (EMT) has emerged as a critical step in the conversion of early stage to aggressive cancer. Employing our previously established stable human cancer cell lines {prostate (LNCaP), breast (MCF-7) and fibrosarcoma (HT1080) cells} over expressing MT1-MMP-GFP (MT1-GFP) as well as GFP expressing control cells (Thromb Haemost **93**:770–8; 2005), we examined the role of MT1-MMP in promoting EMT. Expression of MT1-MMP in all three cell lines promoted cell proliferation in 3 dimensional (3D) cultures, but not in 2D cultures. Enzymatic activity of MT1-MMP is required for enhanced cell proliferation suggesting that cellular degradation of surrounding collagen is a prerequisite. GFP-expressing control LNCaP and MCF-7 cells cultivated in 3D type I collagen gels gradually formed spherical aggregates, whereas cells expressing MT1-GFP displayed a scattered growth pattern. MT1-MMP-induced cell scattering was abrogated by targeted inhibition of either the catalytic domain (TIMP-2) or the hemopexin domain (recombinant PEX) domain of MT1-MMP, indicating a specific role for each domain in cell scattering. Abrogation of MT1-MMP-induced phenotypic changes (fibroblast-like morphology and cell scattering) was reversible upon withdrawal of inhibitors. MT1-MMP transfected LNCaP and MCF-7 cells also degraded the cell–cell adherins junction molecule, E-cadherin. In contrast to epithelial carcinoma (LNCaP, MCF-7), non transfected HT1080 cells, which are mesenchymal in origin, displayed a scattered growth pattern in 3D collagen gels. Transfection of HT1080 cells with MT1-MMP or treatment with high dose TIMP-2, did not alter the scattered growth pattern of HT1080 cells. These results are consistent with experiments demonstrating that mesenchymal-amoeboid cell invasion is independent of MMP activity (Wolf, K., *et al*. J. Cell Biol. **160**: 267–77; 2003). We hypothesize that MT1-MMP degradation of E-cadherin and collagen induce signal transduction pathways leading to mesenchymal-associated morphologic changes and promoting cancer cell migration and proliferation in 3D collagen gels, thus recapitulating EMT. The central role of MT1-MMP in epithelial cancer progression provides a stimulus for development of specific inhibitors as treatment of early stage cancer.

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S14. CROSS-ANALYSIS OF GENE AND miRNA EXPRESSION IN HEREDO-FAMILIAL BREAST CANCERS

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Five to ten percent of all breast carcinomas originate from a hereditary predisposition. Some of them have been associated to mutations in the BRCA1 and BRCA2 susceptibility genes. However, a large number of hereditary breast cancer cases are not accounted by mutations in these two genes and are believed to be due to as yet unidentified breast cancer predisposition genes (BRCAX). Despite intensive effort, their identification has been so far unsuccessful, presumably because of genetic heterogeneity, low penetrance, or recessive/polygenic mechanisms.

We have performed a comprehensive transcriptional profiling analysis on a group of tumor specimens from BRCA patients with the aim to find if the different genetic condition can distinguish BRCA1, BRCA2 and BRCAX cases and is able to explain the heterogeneity of BRCAX families. Moreover, we have integrated mRNA and MiRNA analyses on the same cases to identify MiRNA dependent mechanisms involved in hereditary breast cancer. We have analyzed 50 familial breast cancer cases consisting in 18 samples from patients characterized by carrying germ-line mutations of BRCA1 gene, 11 samples with BRCA2 mutations and 20 samples from BRCAX patients, respectively. As a control, 13 tumors from patients with no family history of breast cancer were similarly analyzed.

Since microarray studies showed that the main clusterization in terms of gene expression profiles of breast cancers follows the status ER+ (estrogen receptor positive) and ER− (estrogen receptor negative), we applied a linear model that adjusted for ER status the sample analysis. After the adjustment, class comparison studies show that only BRCA1 samples exhibited a significant number of differentially expressed genes compared to any of the other hereditary or non-hereditary groups of samples.

Interestingly, BRCAX cases cluster into two distinct subgroups, one mixed up to BRCA2 and sporadic cases, the other closer to BRCA1 samples, and containing only BRCAX cases. The distinct biological identity of the two sub-groups is further supported by the fact that the same bipartition occurs when we used the 6 genes common between our and Hedenfalk et al.'s (PNAS, **100**: 2532–7; 2003) classifier which defines BRCAX subgroups. Clinical characteristics of the tumors show that those belonging to the group containing only BRCAX cases are similar to BRCA1 tumors. Samples mixed to BRCA2 and sporadic cases are more heterogeneous, suggesting a possible involvement of low penetrance genes.

Analysis of the same cases by miRNA expression profile on an array containing 245 miRNA from human and mouse genome highlighted that miRNA deregulation is a frequent event in sporadic breast cancer and distinguishes it from the heredo-familial cases. It is also worth noting that MiRNA analysis confirmed the existence of the two BRCAX sub-groups.

We are now integrating gene expression and miRNA data to identify the targets of the miRNAs found as differentially expressed in the groups in analysis.

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S15. USE OF SPECIALIZED ARRAYS FOR THE DIAGNOSIS OF PANCREATIC TUMOURS

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DNA array technology holds great promises for the improvement of diagnostic procedures in many medical fields. We demonstrate that expression profiling analyses of FNAB samples using