nology to lung preneoplastic lesions.Thus both protein and gene expression profiling can identify molecular classes of non-small cell lung cancers. Profiles of proteins and genes obtained with MALDI-TOF MS and cDNA microarray analysis may lead to a better understanding of human lung cancer development and behavior. The identification of key differentially expressed proteins between individual tumors and between tumors and normal tissues may lead us to identifying novel targets for diagnostic markers and therapeutic intervention.

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Paired-box containing transcription factors as targets for therapy in solid tumors, melanomas and brain tumors

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Paired-box containing transcription factors are crucial regulators of developmental processes. Interestingly, they also seem to play an important role in tumor development as implied by their involvement in specific chromosomal translocations. PAX3 and PAX7, mostly as chimaeric proteins resulting from the fusion with FKHR, a member of the forkhead family of transcription factors, are found in the pediatric tumor rhabdomyosarcoma (RMS). Expression of PAX5 is upregulated via a translocation in B cell lymphomas and PAX8 is involved as a fusion partner in thyroid carcinomas.Furthermore, screening studies done in our laboratory of additional cancer types by RT-PCR and in situ hybridisation revealed PAX3 expression in about 50% of brain tumors and in about 75% of melanomas. Previous experiments in our laboratory have shown that one of the possible oncogenic functions of PAX3/FKHR in RMS is protection from apoptosis. Surprisingly, a similar function was found for the native PAX3 in these tumor cells. Reporter-gene assays demonstrated that the anti-apoptotic function might be mediated through direct transcriptional stimulation of the bcl-xl gene. Additionally, modulation of both PAX and bcl-xl protein levels in tumor cells through antisense oligonucleotides showed that both proteins are functionally important for cell survival, suggesting that PAX3 and PAX3/FKHR might be important targets for therapy in a range of tumor types.To study the influence of PAX3 and PAX3/FKHR on RMS development in more detail, we are currently analysing gene expression of RMS cells and their normal parallel, myoblasts, by means of cDNA microarray technology. Transcriptome analysis may help classify subtypes of RMS on the basis of gene expression profiles, allowing for patient tailored treatment regimes.

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Approaches to structure-based drug discovery for the HSP-90 family

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HSP90a, HSP90b, GRP94 and TRAP1 are a family of cellular chaperone proteins responsible for maturation of a variety of key client proteins involved in cell growth [1]. The last step in the refolding process is ATP-dependent dissociation of the client protein from HSP90 and other chaperones. HSP90s contain an unusually shaped ATP binding cleft that can be inhibited with some selectivity by ansamycin antibiotics such as geldanamycin. Recent trials with geldanamycin derivatives suggest that cancer cells are particularly sensitive to HSP90 inhibition, with the mis-folded client proteins inducing apoptosis and subsequent tumour cytostatis. These studies suggest the HSP90 family may be an appropriate target for anti-cancer drug development. [1] We have determined the crystal structure of members of this family of proteins using X-ray crystallography, in complex with known and novel inhibitors. These structures provide the starting point for series of structure-based drug design approaches. We are using our in-house virtual screening program, RiboDock® to search our large library of 4m compounds for novel ligands that may bind. In addition, we have developed a streamlined fragment-based approach combining NMR and crystallography to identify novel structural moieties that will bind to the ATP binding pocket. These structural studies lay the foundations for the design and development of improved compounds with appropriate drug-like properties as inhibitors.

References

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A protein containing the DHHC domain is upregulated in ovarian carcinomas

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Summary: Ovarian Cancer is still the gynecological tumor with the highest mortality. Early diagnosis and biological characterization of the tumors will be crucial to improve prognosis and individualize therapy of the disease. The aim of this study was to identify genes which are differentially expressed in ovarian carcinoma and encode proteins which could serve as marker proteins or targets for novel biological therapies. Amongst others found by differential display analysis, a cDNA encoding a protein with a conspicuous cysteine-rich motif was found to be overexpressed in ovarian tumors.

Methods: Cells were obtained by taking swabs from ovarian carcinomas as well as from normal epithelium of the distal inner part of the fallopian tube from the same patients, or from ovarian surface epithelium. Samples contained > 95% epithelial cells as judged by quantitative PCR with primers for cDNAs specific for hematopoietic and stroma cells. CDNA of cells microdissected from frozen tissue sections was used for quantitative PCR. Differential display was performed using a modification of Liang's and Pardee's method and a different set of primers. Differential gene expression was confirmed by PCR using gene-specific primers and quantitation in the Agilent Bioanalyser system.

Results: By differential display analysis, a 379 bp cDNA fragment was found to be 4-30 fold overexpressed in 4 out of 5 ovarian tumors analyzed (one borderline tumor and 4 serous carcinomas). Databank searches showed it to encode a member of the DHHC-family of proteins featuring a strictly conserved cysteine-rich domain. The overexpression was confirmed by quantitative PCR using cDNA from tumor as well as microdissected ovarian surface epithelium. Data bank searches revealed a protein from the DHHC family containing a strictly conserved cysteine rich domaine. These proteins are highly expressed in developing tissues in the later stages of organogenesis whereas expression in adult tissues is much more limited.

Conclusions: A cDNA found to be upregulated in ovarian tumors encodes a member of the highly conserved DHHC protein family. The degree of conservation indicates its important role in cellular functions. Its involvement in the pathogenesis of ovarian cancer and its possible use as a prognostic marker have to be further investigated.

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Gene expression profiling of tumor cells with varying levels of stromal involvement: a novel *in vitro* model for studying tumor-stroma interactions

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The importance of stromal cells in tumor progression has been well-recognized but their role in the malignant process has not been fully explored. We have developed a novel, multicellular assay system that utilizes the three primary cell types involved in tumor growth and progression (endothelial, myofibroblasts and tumor cells). Fluorescently-labeled human microvascular endothelial cells and human fibroblasts expressing alpha-smooth muscle actin (myofibroblasts), are cultured in the presence of a tumor cell cylindroid embedded in a layer of Matrigel. After two days, the endothelial cells surround the tumor cell cluster, while the fibroblasts invade the cluster localizing in the center of the malignant cell mass. When endothelial cells are cultured in the presence of fibroblasts, they also invade the tumor cell cluster and co-localize with the fibroblasts. The extent of stromal infiltration into the tumor cell cylindroid depends on the tumor cell type. The breast carcinoma cell line, MDA-MB-231, and melanoma cell lines, MEL624 and A375, have significant stromal invasion, while prostate carcinoma cell lines, PC3 and DU145, fail to demonstrate significant stromal invasion. Serial analysis gene expression (SAGE) profiles of these cell lines identify genes that may be responsible for promoting stromal invasion. These observations support the hypothesis that the interaction between malignant cells, endothelial cells and myofibroblasts is essential in the process of tumor growth and progression. We have also identified potential therapeutic targets for interfering with malignant cell promotion of stromal involvement.