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

Marco A. Pierotti. *Department of Experimental Oncology and Labs., Istituto Nazionale per lo studio e la Cura dei Tumori, Milan, Italy; Cancer Genetic Unit at the FIRC Institute of Molecular Oncology Foundation (IFOM Foundation), Milan, Italy.*

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

Thomas M. Gress. *Philipps University Marburg, Germany.*

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

specialized diagnostic arrays significantly improves the accuracy of diagnosis of suspect masses in the pancreas. We have constructed diagnostic arrays to only contain genes with diagnostic and/or prognostic potential for the classification of pancreatic tissues, augmented with control features. Our results demonstrate that this setup is suitable to produce reliable, reproducible and informative expression profiles of pancreatic tissues and biopsy samples. Expression profiling analysis using the specialized diagnostic array in conjunction with conventional cytology allows the distinction between pancreatic ductal adenocarcinoma (PDAC) and non-malignant diseases of the pancreas with almost 100% diagnostic accuracy. We are currently in the process of analyzing additional tumor entities, such as acinar and neuroendocrine tumors, using both the diagnostic array as well as large scale arrays, in order to develop a multiclass classification system for the comprehensive diagnosis of different malignancies in the pancreas. In addition, we expect further development of the array in combination with careful analysis of clinical patient data to result in the recognition of distinct prognostic gene expression signatures predicting important clinical parameters such as stage of disease, response to therapy, or prognosis. Specialized DNA arrays thus represent valuable new diagnostic tools which can significantly expand the range of information gained in routine diagnostic procedures, thus providing a better basis for decisions on treatment options and setting the stage for therapeutic regimens custom tailored to the individual patient.

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S16. HIERARCHICAL NEURAL NET TECHNOLOGY FOR MOLECULAR STAGING WITH CLINICAL DATA

R. Kates[a,b], H. Allgayer[c], N. Harbeck[b]. [a]*REK Consulting, Germany;* [b]*Technical University of Munich, Germany;* [c]*Department of Experimental Surgery, Universitätsklinikum Mannheim, Ruprecht-Karls-University Heidelberg, Mannheim, Germany.*

Trained neural networks can be used to construct scoring models for molecular staging in cancer. They offer considerable flexibility for representing nonlinear interactions, but because of their flexibility they also tend to require substantial "training" data. It often happens that clinical factors are available in a large collective, but molecular data is available only for a smaller subset. A hierarchical neural net training architecture is presented here. Hierarchical nets are trained in levels, the first level on a large cohort with limited (usually clinical) factors, the second and possibly higher levels on cohort subsets with more (usually molecular) factors, and so on. The scores produced at the first level are treated as "factors" for the second level, and so on. In diseases with distinctly classifiable modes or sites of recurrence (e.g., bone vs. soft tissue in breast cancer), the "competing" risks can be modelled within the neural network architecture. To test the hypothesis that a molecular staging factor might signal the particular relapse mode, one can study significant correlations between factors and "hidden" nodes of a trained neural network. A method is also described for using trained neural nets to generate hypotheses about potential subgroups for molecular staging targets. Applications to breast, colon, and gastric cancer are reviewed.

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S17. BIOINFORMATICS TOOLS FOR MOLECULAR CANCER DIAGNOSTICS ON MICROARRAYS

Jörg Hoheisel. *Functional Genome Analysis, German Cancer Research Center, Heidelberg, Germany.*

In the *Division of Functional Genome Analysis* at DKFZ, we are developing technologies for the identification, description and evaluation of cellular functions and their regulation by producing and processing biological information on a genomic scale. Many chemical and biophysical issues are being addressed in an attempt to understand the underlying procedural aspects, thereby establishing superior analysis processes.

Concerning human material, systems are being developed toward early diagnosis, prognosis and evaluation of the success of disease treatment with an accentuation on cancer. To this end, comparative studies on epigenetic and splice variations, transcription factor binding, transcriptional activity and actual protein expression are under way. Early diagnosis from body fluids is being worked at that is based on the binding of their components to peptide and antibody microarrays.

Combining this data with clinical information permits the definition of patient sub-groups and may provide a robust means for diagnosis and prognosis and lead to the identification of relevant molecular activities. We have established processes – both experimentally and in the area of bioinformatics – to deal with this challenge. The combination will not only occur in silico, since the various molecular levels affect each other extensively. Soon, current in silico systems biology will translate into ever more complex experimental set-ups that permit an evaluation of a biological issue in a systemic experimentation. Similar to research in physics, an iterative interaction of theoretical and experimental systems biology will yield important insights into function, providing the archetypal platform for an eventual model of a cell.

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S18. CONVENTIONAL STATISTICAL METHODOLOGY IN LARGE SCALE PROFILING

Rainer Spang. *Max Planck Institute for Molecular Genetics, Berlin, Germany.*

A plethora of computational methods for the diagnosis of cancer using gene expression profiling has been suggested. This might come as a surprise, since diagnosis appears to be a straightforward classification problem. What can be done with methods from standard statistics textbooks? Clearly, the problem is the large number of genes on the arrays. Including them all in a classification model leads to saturation of the model.