CONCLUSION: This is the first comprehensive study describing how tenascin-C promotes tumorigenesis in vivo. Our data suggest that tenascin-C promotes several events leading to metastasis, that will be described in detail. This knowledge is important to combating tenascin-C actions in cancer.

33 Oral

Breast tumor environment inhibits human plasmacytoid dendritic cell functions

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Background: A retrospective analysis performed with primary breast carcinomas has reported that the infiltration of plasmacytoid Dendritic Cells (pDC) is associated with an adverse clinical outcome, suggesting that pDCs are involved in breast cancer progression. Indeed the tumor micro-environment may modulate pDC functions for the induction of tumor growth or facilitate the tumor progression by interfering with the immune response.

Material and Methods: To understand the negative influence of the breast tumor environment on human pDC functions, we developed three complementary strategies: 1) an ex vivo phenotypic and functional analysis of breast tumor-infiltrating pDC, 2) an in vitro study of control pDC co-cultured with breast tumor-derived supernatants, and 3) an in vivo model of breast tumors xenograft infiltrated by a pDC cell-line (Gen2.2) in SCID/NOD/b2m-/- mice.

Results: Our first ex vivo studies showed that human breast tumor-infiltrating pDC (Ti-pDC) have an activated phenotype and a lower capacity to produce IFNa in response to Toll Like Receptors (TLR) ligands. We also observed in vitro that breast tumor supernatants specifically inhibited IFNa secretion by activated control pDC. Interestingly as observed with Ti-pDC or normal pDC cultured in presence of breast tumor supernatants, tumor-injected pDC GEN2.2 have mostly an impaired TLR9 responsiveness. Other in vitro studies have also shown that pDC in presence of breast tumor supernatants keep their capacity to induce T-cell proliferation but direct those T-cells to produce high amounts of an immunosuppressive cytokine IL-10. Soluble factors such as TNFa and TGFb which are present in the breast tumor environment seem to be involved in the functional alteration of pDC. Indeed the use of blocking antibodies against TNFa and TGFb restored the production of IFNa by activated pDC in presence of tumor supernatants in vitro. The effect of TNFa and TGFb on the capacity of pDC to induce IL-10-producing Tcells is under investigation.

Conclusions: Our results suggest that the breast tumor microenvironment subverts pDC function in order to maintain tumor tolerance. Further studies are ongoing in our xenograft model in order to validate some new therapeutic approaches that are based on the reversion of the functional inhibition of Ti-pDC to induce an effective antitumor immunity in breast cancer.

34 Oral

Molecular subclassification of breast carcinomas based on aCGH, gene expression, IHC and ploidy - relevance for clinical outcome

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Identification of well defined molecular subgroups of carcinomas is important for identification of novel therapy targets, for prediction of response and for improvement of prognostication. A molecular taxonomy for breast cancer based on expression profiling identified five subgroups: luminal A, luminal B, basal-like, ERBB2+, and normal-like (Perou et al. Nature 2000, Sørlie et al. PNAS 2001). Based on genomic alterations Hicks et al. (Genome Res 2006) identified three different patterns of alterations, “simplex”, ”complex” and “firestorm”. The aim of this study was to explore both genomic, gene expression and protein data from early stage breast carcinomas to develop a combined and robust classifier that distinguishes between distinct biological subgroups with clinical relevance.

Tumor tissue from 137 early stage breast cancer patients was analyzed for genomic alterations by high-resolution aCGH, HER2 amplification by FISH, TP53 mutation by sequencing, expression subclasses by DNA microarrays, and ploidy and protein expression using tissue micro arrays (TMA). We designed a CGH classifier based on known genomic alterations characteristic of the intrinsic subgroups, and applied a mathematical algorithm on the aCGH data that defined loss and/or gains of whole arms in addition to more complex alterations (“firestorms”).

Based on the developed CGH classifier, a luminal (48%), non-luminal (20%), a mixed (11%) and an unclassified (20%) group was identified. The luminal subgroup was dominated by loss or gains of whole chromosome arms. More than half were diploid, the rest aneuploid. Most tumors were ER+ (82%) and only 7% HER2+. 62% of this group was lumA by gene expression, the remaining lumB, ERBB2+ or normal-like. The non-luminal subgroup showed more complex genomic alterations, 50% were basal-like and 44% were either luminal B or ERBB2+ by gene expression. 81% had TP53 mutations. This group could further be stratified by HER2 status; the HER2+ were aneuploid and either ERBB2+ or lumB by gene expression; the HER2- were CK5/6+ and/or 17+ by IHC, either diploid or aneuploid, and basal-like by gene expression. Samples in the mixed and the unclassified subgroups were mostly aneuploid, and all expression subclasses were represented.

Our data from combined molecular profiling identify relevant clinical subgroups with different outcome. These results have to be validated in a larger cohort.

35 Oral

Activation of alternative HER receptors mediates resistance to EGFR tyrosine kinase inhibitors in breast cancer cells

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The response rate to EGFR inhibitors may be poor and unpredictable in cancer patients with EGFR expression itself being an inadequate response indicator. There is limited understanding of the mechanisms underlying this resistance. Here we have provided a molecular mechanism of alternative HER receptor activation (ErbB receptor family members) in mediating resistance to EGFR TKIs in breast cancer cells. Using both Förster Resonance Energy Transfer (FRET) which monitors in situ HER receptor phosphorylation as well as classical biochemical analysis, we have shown that the specific tyrosine kinase inhibitors (TKIs) of EGFR (HER1), AG1478 and Iressa (Gefitinib) decreased EGFR and HER3 phosphorylation through the inhibition of EGFR/HER3 dimerization. Consequent to this, we demonstrate that cleavage of HER4 and dimerization of HER4/HER2 occur together with reactivation of HER3 via HER2/HER3, leading to persistent HER2 phosphorylation in the now resistant, surviving cells. These drug treatment–induced processes were found to be mediated by the release of ligands including heregulin and betacellulin that activate HER3 and HER4 via HER2. Whereas an anti-betacellulin antibody in combination with Iressa increased the anti-proliferative effect in resistant cells, ligands such as heregulin and betacellulin rendered sensitive SKBR3 cells resistant to Iressa. These results demonstrate the role of drug-induced autocrine events leading to the activation of alternative HER receptors in mediating resistance to EGFR tyrosine kinase inhibitors (TKIs) in breast cancer cells, and hence specify treatment opportunities to overcome resistance in patients.

36 Oral

Large scale comparative proteomic study of accessible vascular proteins in mouse liver metastases and normal liver

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INTRODUCTION: The aim of our study is the identification of tumour associated antigens (TAA) localized at newly formed blood vessels or in the surrounding stroma as a tool for the development of novel antibody-based therapy with special focus on the metastatic process.

METHODS: Three murine cancer cell lines metastasizing to the liver (M5076, Colon38 and SL4) were injected into C57BL/6 mice and tested for their metastatic potential. Tumour-bearing and healthy mice were subjected to terminal perfusion with a reactive ester derivative of biotin (Sulfo-NHS-LC-Biotin) in order to chemically modify accessible membrane and extracellular matrix proteins from the bloodstream. Biotin labelled proteins are purified on streptavidin resin, trypsinized on resin and subsequently analysed by RP-nano-HPLC and MALDI-TOF/TOF procedures. Peptides are identified by the Mascot software and relatively quantified by 2D-peptide maps using the DeepQanTR software.

RESULTS: Three different syngenic mouse models were set up in order to reproduce and study the complex hepatic metastasis process. Namely, M5O76 (mouse reticulum sarcoma), Colon38 (mouse colon carcinoma) and its highly metastatic variant SL4. Mice were then subjected to the in vivo biotinyation technique and biotinylated organs were excised for further analysis. Successful biotinylation of vascular structures was assessed by histochemical analysis using streptavidin-alkaline phosphatase complex.

Results show a strong staining of blood vessels with some degree of diffusion into the surrounding stroma of both normal and metastatic tissue. Organs were homogenized and processed as previously described.

A total of 36 samples was analyzed by mass spectrometry resulting in the identification of 9481 different peptides which could be clustered to 1902 proteins. More than 500 proteins were exclusively identified in tumor samples but neither in healthy livers nor in negative controls. The choice of candidate marker proteins, the expression of suitable domains and the selection of monoclonal antibodies by phage display technology is ongoing.

CONCLUSIONS: In this study we show successful chemical modification of membrane proteins of selected mouse models which closely mimic the metastatic spread of colorectal cancer. Our proteomic results allow for the first time the creation of comprehensive tissue specific protein lists which promises to identify novel TAA easily reachable by antibody derivatives for the therapy and diagnosis of metastatic colorectal carcinoma.

06 July 2008 14:35 - 16:05

PRESIDENTIAL SESSION
APPLIED BIOSYSTEMS – EACR 40TH ANNIVERSARY RESEARCH AWARDS

Cancer genetics

38 Oral

Screening 101 renal cancers for somatic mutations in 3,726 genes

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Around 190,000 new cases of renal cancer are diagnosed in the world each year. Despite the frequency of this type of cancer, little is known about the genetic events involved in sporadic renal carcinoma. One notable exception is the VHL gene which has a deleterious somatic mutation in around two thirds of all clear cell renal cancers. A recent screen for somatic mutations in protein kinases failed to identify any genes with substantial evidence for involvement in the disease.

We report here results of sequencing the coding exons of 3,726 genes in a collection of 101 DNA samples from 96 primary cancers and 5 renal cancer cell lines, each with a matched normal DNA sample. The gene set was derived from several sources including gene families where one member has previously been shown to be mutated in human cancer, genes resident in amplified regions of human cancer genomes, and genes found to be targeted in mouse mutagenesis screens for cancer.

Over 300 somatic mutations were uncovered in the course of this screen. Consistent with our previous analysis of protein kinase gene mutations, renal cancer mutation prevalence is towards the lower end of the spectrum when compared with cancers derived from other tissues. The number of mutations found varied significantly between individual cancers. Over 200 genes were found to have at least one somatic mutation and in most cases these genes harboured only one or two somatic mutations. The mutation spectrum in renal cancers was noted to be different to other cancer types in several instances. Deletion mutations, often localised to poly-nucleotide tracts, are almost 5 times more prevalent than observed in other cancer types we previously screened (mainly lung, breast and melanoma cell lines). The prevalence of C:G>T:A type substitutions was approximately 20% lower than observed in these other cancers while T:A>C:G type substitutions were around 10% more prevalent.

The scale of this sequencing project has allowed both the mutation prevalence and mutation spectrum of individual renal tumours to be studied in depth and allowed comparisons between primary tumours and renal cell lines. It was interesting to note that in contrast to other tumour types (e.g. breast or melanoma) mutation prevalence and spectrum in individual renal cancers was relatively homogeneous. One notable exception was an apparent deletion phenotype observed in some primary renal tumours and cell lines. Further investigation of somatically mutated genes identified in this screen will likely provide insights into renal cancer development.

39 Oral

The impact of defined Brca1 mutations on tumor development, drug response and acquired resistance

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Women with heterozygous germline mutations in BRCA1 have a strongly increased lifetime risk of developing breast and/or ovarian cancer. To study the role of BRCA1 and in breast tumorigenesis, we have developed a conditional mouse model (K14cre;Brca1$^{F/F}$;p53$^{F/F}$) for BRCA1-associated breast cancer. Intervention studies in this mouse model have shown that BRCA1-deficient tumors are more sensitive to platinum drugs than to other conventional chemotherapeutic agents. Strikingly, the Brca1$^{D/D}$;p53$^{D/D}$ mouse mammary tumors (which lack Brca1 exons 5-13) do not become resistant against platinum drugs, suggesting that (partial) BRCA1 function is required for platinum resistance. We therefore hypothesize that genetic reversion of BRCA1 alleles with truncation mutations may underlie the induction of platinum resistance. In line with this notion, preliminary clinical data suggest that in BRCA1 mutation carriers with advanced ovarian cancer (who receive systemic therapy with carboplatin) the survival time is also affected by the type of founder mutation, since the BRCA1^{5382insC} founder mutation appears to be associated with a relatively favorable survival time, compared to the BRCA1^{185delAG} mutation. To investigate whether different founder mutations are indeed causally related to differences in sensitivity to platinum-based chemotherapy, in vitro cytotoxicity studies and in vivo intervention studies with platinum drugs will be performed, using cell lines and mice carrying these specific BRCA1 mutations. In case resistance to platinum drugs is observed, it will be investigated whether resistance occurs via genetic reversion of BRCA1 and/or via other mechanisms.

Platinum resistance is a serious problem in the treatment of BRCA-related cancers. This research could reveal differences in sensitivity to platinum drugs of different BRCA1 mutations. This insight could lead to various treatment strategies for carriers of different BRCA mutations and thereby hopefully to a better survival.

40 Oral

A genome-wide association study of tag SNPs identify five novel colorectal cancer susceptibility loci

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It has been estimated that genes of low-penetrance are involved in more than a third of all colorectal cancers (CRCs). To identify novel CRC susceptibility loci, we carried out a multi-stage genome-wide association study using two large British case-control cohorts. To maximise the power of our investigation, we decided to enrich the discovery phase (Phase 1) with cases that had a strong family history of colorectal neoplasms and with "hypernormal" cancer-free controls. In Phase 1, we genotyped 550,163 tagging SNPs in 940 cases and 965 controls. Three SNPs approaching genome-wide significance after Phase 1 (rs6983267, rs4939827 and rs4779584) were examined in three replication sample sets comprising 7,473 cases and 5, 984 controls. Across the four sample sets, the associations between these three SNPs remained statistically significant, confirming the existence of susceptibility loci at 8q24.21, 15q13.3 and 18q21.1. To identify additional susceptibility loci, about 40,000 SNPs showing association at P<10-2 in Phase 1 were examined in a second phase using 2,873 sporadic CRC cases and 2,871 population controls. 11 SNPs retaining association at P<10-4 were examined in a third phase of the study that comprised 4,287 cases and 3,743 controls. After this latter phase, two SNPs were taken forward for validation in the four and last phase of our investigation. In Phase 4, we examined 10,731 CRC cases and 10,961 controls from 8 centres from Europe and Australia. In addition to the three previously identified susceptibility loci, we identified two novel associations at 10p14 and 823.3. These five novel susceptibility loci tag potentially interesting candidates that include POU5F1P1, SMAD7 and EIF3S3. Our investigation demonstrated the existence of common susceptibility alleles in CRC predisposition.

41 Oral

Genetic variants of miRNA sequences and non small cell lung cancer survival

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Background: Recent evidence indicates that small, non-coding RNA molecules, called microRNAs (miRNAs), function as tumor suppressors or oncogenes. Mutations, mis-expression or altered mature miRNA processing are implicated in carcinogenesis and clinical behavior.

Materials and methods: We conducted a systematical survey of common SNPs in miRNAs and their surrounding regions and evaluated the associations of four SNPs in pre-miRNAs with non small cell lung cancer (NSCLC) survival.

Results: We found that rs11614913 in hsa-mir-196a-2 was significantly associated with NSCLC survival in the recessive genetic model. Stepwise