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

Cox proportional hazard regression analysis revealed that rs11614913 variant homozygous genotype CC was a significantly unfavorable prognostic factor of NSCLC [Hazard ratio (HR) =1.76, 95% CI=1.34-2.32)]. In the genotype-phenotype correlation analysis using 23 tumor tissue samples, rs11614913 variant homozygote CC was associated with a significantly increased mature hsa-mir-196-a expression in the recessive model (P = 0.037), which might be due to an enhanced processing of pre-hsa-mir-196-a to its mature form. Conclusions: rs11614913 might be an independent prognostic biomarker for NSCLC. Further characterization of miRNA SNPs may open new avenues for cancer biological studies and therapeutic interventions.

42 Oral

ATM in breast cancer susceptibility - results of a pooled analysis of case-control mutation screening data

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The susceptibility gene for Ataxia telengiectasia, ATM, has been established as an intermediate-risk breast cancer susceptibility gene. However, the answer to the question "what sort of sequence variation in ATM confers increased risk of breast cancer" has been controversial. To address this question, we have pooled available ATM mutation screening data and then carried out a joint analysis of truncating variants, splice junction variants, and rare missense substitutions. A total of 1,729 breast cancer cases and 941 controls from 13 published studies were included in our pooled analysis. The analysis of rare missense substitutions was accomplished using the missense analysis program Align-GVGD with our improved classifier and a ATM protein multiple sequence alignments containing ATM sequences from human to sea urchin. We found that a trend test, incorporating both truncating plus splice junction variants and several grades of rare missense substitutions outperformed simple consideration of truncating plus splice junction variants alone. We also found significant evidence of risk both in truncating plus splice junction variants and in the in silico predicted highest-risk grade of missense substitutions. Taken together, these results led us to two conclusions: (1) careful analysis of missense substitutions will have real utility in case-control mutation screening projects, and (2) the attributable fraction, for risk of breast cancer, of rare missense substitutions in ATM is approximately equivalent to that of truncating and splice junction variants. Resequencing the entire coding sequence of ATM in 650 familial cases and 650 controls from 4 different populations is currently ongoing in the Genetic Susceptibility Group at IARC. Combined analysis of truncating and splice junction variants, and rare missense substitutions will be performed on our sample set, as a validation step of this approach.

06 July 2008 17:30 - 18:30

PLENARY LECTURE

AICR Lecture

43

Rho GTPases, actomyosin contractility and cell migration

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Cell movement plays a central role in tumour metastasis and in the generation of tumour blood supply and other stromal functions. We seek to determine how signalling pathways determine movement of tumour cells and of associated non-neoplastic cells such as endothelial cells. We have shown that tumour cells move in a 3-dimensional environment in two very different ways termed "elongated-mesenchymal" and "rounded-amoeboid". While the "elongated-mesenchymal" mode of movement in 3D is akin to movement of mesenchymal cells in 2D, "rounded-amoeboid" movement is only seen in 3D. These different modes of movement have very different requirements for small GTPase signalling pathways. The "rounded-amoeboid" form of movement has an absolute requirement for Rho-Rho-kinase signalling to drive high levels of actomyosin contractility, whereas Rho-kinase is not essential for mesenchymal movement. A major focus of our current studies is to determine how different signalling pathways interact to determine cell movement. A key determinant of mode of cell movement is the degree of actomyosin contractility. We are studying how actomyosin contractility is regulated by GTPase and kinase signalling pathways, and how the degree of contractility influences cell behaviour.

POSTER SESSION

Cell and tumour biology 1

44 Poster

Reactivation of telomerase activity in telomerase-deficient human cells by the pseudourydine synthase domain of dyskerin

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A dyskerin internal fragment, isolated in a genetic suppressor element (GSE) screening for cisplatin resistance, named GSE24.2, increases telomerase activity in different cellular systems. GSEs are biologically active gene fragments that encode either peptides or inhibitory antisense RNAs and act dominantly upon expression in mammalian cells. The GSE24.2 contains the pseudourydine synthase domain of the dyskerin, a protein that forms part of the telomerase complex. This protein is mutated in patients with X-linked dyskeratosis congenita (X-DC) resulting in greatly reduced levels of telomerase activity. GSE24.2 expressing cells showed impaired telomerase inhibition after cisplatin or chemical telomerase inhibitors treatment. The promoter of telomerase component hTERT was constitutively activated in GSE24.2 cells in a c-MYC expression-dependent manner. Furthermore, expression of GSE24.2 in cell lines derived from X-DC patients and VA13 cells increases hTERT and hTR RNA levels and recovers telomerase activity. Finally, expression of GSE24-2 was able to rescue X-DC fibroblasts from premature senescence. These data demonstrate that this internal domain of dyskerin plays an important role in telomerase maintenance after cell insults, such as cisplatin treatment and in telomerase-defective cell lines. The expression of the dyskerin fragment showed a dominant function in X-DC cells, providing the basis for a therapeutic approach to this disease.

45 Poster

A Twist – Snail axis critical for TrkB-induced metastasis

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Metastasis corresponds to the biggest cause of death of cancer patients. A better understanding of the molecular mechanisms mediating metastasis may help to tailor better drugs for anticancer therapy in the future. To identify novel metastasis-associated genes, we previously set up a functional, genome-wide screen for genes that could suppress anoikis (cell-detachment induced apoptosis). Anoikis is thought to provide a physiological barrier against the metastatic spread of tumor cells. In this screen, we identified the neurotrophic receptor kinase TrkB as a potent suppressor of anoikis. Consistent with our hypothesis, TrkB-expressing cells formed highly invasive and metastatic tumors when injected into nude mice. A structure-function analysis indicated that all of the oncogenic and metastatic properties strictly depended on TrkB's kinase activity. As TrkB is overexpressed in various human malignancies, it may represent a potential target for anticancer therapy.

Expression of TrkB in epithelial cells induced loss of intercellular adhesion and a striking change in cell morphology, reminiscent of epithelial to mesenchymal transition (EMT). In line with the hallmarks of EMT, we observed a downregulation of E-cadherin upon TrkB expression and an induction of the basic helix-loop-helix transcription factor Twist. Twist is a known mediator of EMT and a metastasis gene. We show by RNAi that Twist is required for TrkB-induced loss of E-cadherin and anoikis suppression, as well as for the growth of subcutaneous tumors in nude mice. To further investigate the function of Twist, we searched for potential downstream effectors. Studies in Drosophila suggested that Twist induces the zinc finger transcription factor Snail, a developmental gene associated with poor prognosis in human breast and other cancers. Indeed, Twist and TrkB each induced Snail. Further functional studies showed that Snail is acting downstream of Twist also in mammalian cells. Furthermore, RNAi-mediated knockdown of Snail impaired the loss of E-cadherin and anoikis suppression by TrkB. Snail knockdown did not affect tumor growth of TrkB expressing cells in nude mice. Instead, it specifically impaired the formation of lung metastases. In conclusion, our data suggest that TrkB signaling activates a Twist – Snail axis that is critically required for metastasis. This