prioritize and focus on evolutionarily conserved events that are more likely to be biologically important. Examples of such approach will be described.

06 July 2008 12:45 - 13:45

YOUNG CANCER RESEARCHER'S WORKSHOPS

How to be effective in applying for fellowships

06 July 2008 13:45 - 14:35

AWARD LECTURE

Young Cancer Researcher's Award

37 Oral

SPAR1 is an anti-recombinase that impacts on genome stability and cancer

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DNA double-strand breaks represent a major threat to genome integrity and are predominantly repaired by homologous recombination (HR). Unscheduled or excessive HR can also lead to gross chromosomal rearrangements characteristic of cancer cells, but the mechanisms that restrain HR remain poorly understood.

Yeast Srs2 and E. coli UvrD are related helicases that suppress aberrant recombination by disrupting a specific step in HR, however functional homologues are not obviously conserved in higher eukaryotes. We therefore performed a genetic screen in C. elegans to identify uncharacterised helicases that are synthetic lethal in combination with C. elegans BLM mutants, based on the srs2 sgs1 (BLM) synthetic lethality observed in yeast. This screen identified a novel helicase, SPAR-1 that is conserved from C. elegans to humans and exhibits many of the genetic and biochemical hallmarks of yeast Srs2. Genetic analysis has revealed that C. elegans spar-1 mutants are also synthetic lethal with mus-81 and a distinct group of non-replicative helicases: BLM, FANCJ and RECQ5, but not with WRN. Additionally, the lethality in all four double mutant combinations results from an accumulation of toxic recombination intermediates. C. elegans spar-1 mutants and SPAR1 deficient human cells are also hyper-recombinogenic and exhibit exquisite sensitivity to interstrand cross-links (ICL) that block replication forks. SPAR1 knockout mice die between days 10 and 11.5 due to dramatic genome instability and rapid telomere loss and Human SPAR1 is over-expressed in gastric tumours. Collectively, our work suggest that SPAR1 acts as suppressor of aberrant recombination.

Further support for an anti-recombinogenic function for SPAR1 has come from biochemical studies. Human SPAR1 co-purifies with the critical recombinase Rad51, and is recruited to replication forks via interaction with PCNA. Purified Human SPAR1 can also actively disassemble post-synaptic recombination intermediates in an ATP-dependent manner. Our data indicate that the phenotypes observed in C. elegans, mice and human cells are caused by a failure to counteract inappropriate or persistent recombination intermediates. Furthermore, we suggest that promiscuous disassembly of recombination intermediates is the underlying cause of the genome instability of SPAR1 over-expressing cancers and propose a potential therapy for treating these cancers with a drug currently in clinical trials.

06 July 2008 14:35 - 16:05

PRESIDENTIAL SESSION
APPLIED BIOSYSTEMS – EACR 40TH ANNIVERSARY RESEARCH AWARDS

Signalling and tumour environment

31 Oral

Rac activation and inactivation control plasticity of tumour cell movement

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Background: One of the major discoveries of the last two decades has been the identification of Rho-family GTPases as key regulators of actin dynamics and cell movement. The activity of these GTPases is controlled by activators, guanine nucleotide exchange factors (GEFS) and inactivators, GTPase accelerating proteins (GAPs). How these GEFs and GAPs work together to regulate cell behaviour is a key issue in biology.

Materials and Methods: We carry out the first systematic screen of all known human GEFs and GAPs for Rho-family GTPases.

Results. We identify a GEF-GAP signalling module controlling Rac activity that controls the movement of metastatic melanoma cells. We show that a Rac-GEF interacts with an adaptor protein, that was recently shown to be up-regulated in human tumours and in metastases in a genetically engineered mouse model of melanoma. We show that the complex between the adaptor and the Rac GEF mediates the activation of Rac for cell movement. However, tumour cells can adopt two different modes of movement; a mesenchymal mode where cells have an elongated polarised morphology and an amoeboid mode where cells have a rounded morphology. We show that a series of human melanoma cell lines when cultured on a deformable collagen matrix consist of varying proportions of cells moving in mesenchymal and amoeboid fashions and importantly we show that individual cells within a culture convert between these two different modes. Significantly we show that this inter-convertibility is reciprocally controlled by Rac and Rho. Rac activation through the Rac GEF drives mesenchymal movement and suppresses amoeboid. Rho through activating Rho-kinase activates a Rac-GAP suppressing Rac activation and thereby permitting the high levels of actomyosin contractility required for amoeboid movement. Significantly we show that the expression of the GEF and the GAP determines the way in which different melanoma cells move.

Importantly the data we present is highly relevant to consideration of tumour cell movement in vivo. The biological properties of these different forms of movement provide cells with the ability to cope with different environments in vivo. Mesenchymal movement may be the most fit for rigid tissue environments that require extra-cellular proteolyisis while amoeboid movement in more deformable environments is rapid and its associated high actomyosin contractility provides cells with mechanical strength to deal with shear forces such as following entry in to the blood supply.

Conclusion: Our work leads to the important prediction that tumour cells that can exploit alternative modes of movement may be the most metastatic and that therapies targeting metastasis will have to block both forms of cell movement.

32 Oral

Tumorigenesis-promoting events and signaling by tenascin-C

K. Lange[1], M. Kammerer[1,2], Y. Jia[1,2], A.C. Feutz[1], G. Christofori[1], G. Orend[1,2]
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BACKGROUND: The ECM component tenascin-C is highly expressed in most solid tumors. Its high expression correlates with a bad survival prognosis in patients with several cancers. Results from cell culture experiments support a role of tenascin-C in enhancing tumor cell proliferation, promoting angiogenesis, invasion and metastasis. We showed that tenascin-C induces cell rounding, which may enhance proliferation and migration, by two mechanisms. Tenascin-C counteracts the tumor cell proliferation-suppressing effect of fibronectin by blocking the integrin alpha 5 beta 1/syndecan-4 complex. This caused cell rounding (Orend et al., 2003, Oncogene 22, 3917) and stimulated tumor cell proliferation (Huang et al., 2001, Cancer Res. 61, 8586) by activation of oncogenic Wnt and MAPkinase signaling (Ruiz et al., 2004, Cancer Res. 64, 7377). Tenascin-C also stimulated endothelin receptor type A (EDNRA) expression, and signaling through EDNRA maintained cell rounding (Lange et al., 2007, Cancer Res. 67, 6163). By using knockdown and over-expression studies, we identified paxillin, RhoA and TM1 as critical targets of cell rounding by tenascin-C downstream of syndecan-4 and EDNRA (Lange et al., 2007, Cancer Res. 67, 6163).

MATERIAL & METHODS: To determine a potential tumorigenesis-promoting effect of tenascin-C in vivo, we generated transgenic mice that ectopically express human tenascin-C in the pancreatic islets. Tenascin-C-transgenic mice, that are apparently healthy and fertile, exhibit normal development of the pancreas, but showed enhanced angiogenesis in the pancreatic islets. Next, we crossed RipTNC mice with tumor-prone RipTag2 (RT2) mice, that develop insulinomas due to ectopic expression of the SV40T-antigen and compared tumorigenesis in RT2/TNC and RT2 mice.

RESULTS: Double transgenic RT2/TNC mice experience more frequent and earlier death incidences than RT2 mice. RT2/TNC mice exhibit several signs of enhanced tumor progression, such as the appearance of local and distant metastasis.

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.