repression of Id1 and Id3 levels inhibiting the capacity of cells to initiate tumours. In addition, high CD44 and Id1 levels is a poor prognosis factor in GBM patients. Furthermore, our results have clear implications on the clinical development of TGFβ inhibitors as compounds targeting GSCs.

41 NOTCH2 in breast cancer: association of SNP rs11249433 with gene expression in ER-positive breast tumours without TP53 mutations

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Background: A recent genome-wide association study (GWAS) has identified a single nucleotide polymorphism (SNP) rs11249433 in the 1p11.2 region as a novel genetic risk factor for breast cancer, and this association was stronger in patients with estrogen receptor (ER)-positive versus ER-negative cancer.
Results: We found evidence of a functional relationship between SNP rs11249433 and the expression of the *NOTCH2* gene located in the 1p11.2 region. Examined in 180 breast tumours, the expression of *NOTCH2* was found to be lowest in tumours with *TP53* mutations and highest in *TP53* wild-type/ER-positive tumours ($p = 0.0059$). In the latter group, the *NOTCH2* expression was particularly increased in carriers of the risk genotypes (AG/GG) of rs11249433 when compared to the non-risk AA genotype ($p = 0.0062$). This effect is either tumour or tissue-specific since rs11249433 was not associated with *NOTCH2* expression in blood samples from 302 breast cancer patients and in 76 normal breast tissue samples. We also identified the first possible dominant-negative form of *NOTCH2*; a truncated version of *NOTCH2* consisting of only the extracellular domain.
Conclusion: This is the first study to show that the expression of *NOTCH2* differs in subgroups of breast tumours and by genotypes of the breast cancer-associated SNP rs11249433. The NOTCH pathway has key functions in stem cell differentiation of ER-positive luminal cells in the breast. Therefore, increased expression of *NOTCH2* in carriers of rs11249433 may promote development of ER-positive luminal tumours. Further studies are needed to investigate possible mechanisms of regulation of *NOTCH2* expression by rs11249433 and the role of *NOTCH2* splicing forms in breast cancer development.

42 A therapeutic sphingosine 1-phosphate antibody inhibits intratumoural hypoxia and sensitizes to chemotherapy in prostate cancer animal model

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Background: Hypoxia triggers the activation of signaling pathways promoting neovascularization, metastasis, increased tumour growth, and resistance to treatments. The activation of the transcription factor HIF-1a has been identified as the master mechanism of adaptation to hypoxia. We recently identified the sphingosine kinase 1/sphingosine 1-phosphate (SphK1/S1P) pathway as a new modulator of HIF-1a activity under hypoxia in multiple cancer cell models including prostate cancer (Ader et al, Cancer Res, 2008). S1P elicits various cellular processes including cell proliferation, cell survival, or angiogenesis. S1P is believed to exert most of its actions as a ligand for a family of five cognate G protein-coupled receptors to elicit paracrine or autocrine signaling cascades. We have suggested that inhibiting SphK1/S1P signaling, which is up-regulated under hypoxia, may help normalizing the tumour microenvironment and increase sensitivity to radiation and chemotherapy, in the broader concept of "normalization of tumour vessels" as tumour oxygenation is known to enhance response to chemotherapy and radiation (Ader et al., Cancer Res, 2009).
Methods: Quantitation of intratumoural hypoxia and angiogenesis, and treatment efficacy (primary tumour, metatasis dissemination) using an orthotopic (o.t) xenograft model of fluorescent hormone refractory prostate cancer cells.
Results: We first provide in vitro evidence that inhibition of the S1P exogenous signaling, through pharmacological inhibition of its receptors or by taking advantage of a monoclonal antibody neutralizing S1P, blocks HIF-1a accumulation and its transcriptional activity in prostate cancer cells exposed to hypoxia. Second, using an o.t model of prostate cancer, we show that an anti-S1P antibody inhibits intratumoural hypoxia and modifies vessel architecture within 5 days of treatment. Third, we show for the first time that an anti-S1P strategy sensitizes to docetaxel, the 'gold standard' treatment for hormone-refractory prostate cancer. A 5-day anti-S1P antibody pretreatment markedly sensitizes to docetaxel in an o.t. PC-3/green fluorescent protein model established in nude mice. The combination anti-S1P antibody together with docetaxel was not only accompanied by a smaller primary tumour volume compared to docetaxel treatment, but also significantly reduced the occurrence and number of metastases.
Conclusion: These data establish the proof-of-concept that blocking the exogenous action of S1P reduces intratumoural hypoxia and sensitizes to chemotherapy in prostate cancer animal model.

43 Estrogen receptor alpha is upregulated and metastasis inhibited in a murine breast cancer model following treatment with the novel Wnt-5a derived-hexapeptide, Foxy-5

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Background: Breast cancer remains the most common female cancer worldwide, and mortality from metastatic disease remains a major public health issue. Patients with tumours negative for the nuclear hormone receptor, estrogen receptor (ERα), have a particularly poor prognosis, partly due to their lack of response to current endocrine treatments. Expression of Wnt-5a in tumours is associated with better patient outcome, and reduced migration in breast cancer cell lines [1]. We have previously shown that loss of Wnt-5a is associated with loss of ERα in patient breast cancer material, and that the generation of Wnt-5a signalling upregulates ERα in ERα negative breast cancer cell lines and renders them responsive to the selective estrogen receptor modulator, Tamoxifen [1,2].
Materials and Methods: A Wnt-5a derived hexapeptide, termed Foxy-5, has been developed and shown to possess Wnt-5a signalling properties [3]. Here, we utilised the 4T1 murine metastatic breast cancer model that is negative for both ERα and Wnt-5a. These highly aggressive breast cancer cells were inoculated into the mammary fat pad of Balb/C mice at day 0. Following the development of palpable tumours (day 8), 40 ug of Foxy-5 or a Scrambled control peptide, or PBS alone was administered to animals intraperitoneally every 2 days, until the conclusion of the experiment. Primary breast tumours and metastatic organs were harvested from sacrificed animals and nucleic acid extracted for qPCR and bisulphite genomic sequencing (BGS). Immunohistochemistry (IHC) was used to determine expression of key genes, and the area of individual metastases measured on H&E stained sections.
Results: Foxy-5 administration significantly reduced metastasis to the lungs, even with the treatment delayed until after the detection of primary tumours, to mimic the clinical situation. Epigenetic and qPCR analysis demonstrated that Foxy-5 treated tumours re-express ERα, and that this occurred in parallel with a reduction in methylation of the ERα promoter. We are now actively investigating the feasibility of combinatorial therapy with Foxy-5 and Tamoxifen as a future treatment possibility for ERα negative breast cancer patients, utilising different metastatic mouse models.
Conclusions: Foxy-5 has exciting potential as a new therapy for breast cancer patients due to its ability to address two of the most important aspects of cancer associated mortality − non response to endocrine therapy and metastasis.

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44 Development of diagnostic and therapeutic aptamers against enzymes crucial for tumour development and metastasis

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Background: A variety of enzymes are crucial in tumour development and metastasis. Aptamers are a particularly interesting targeting modality with a unique ability to selectively and specifically bind to their target in diagnostic platforms and therapeutic applications. With the support of the EACR and ECCO through a Mike Price fellowship, we have raised aptamers against human kallikrein 6 (KLK6) and heparanase (Hpa1), two enzymes of diagnostic and therapeutic value against a variety of cancers.
Material and Methods: KLK6 was produced in *pichia pastoris* systems and chromatography purified, at the University of Patras, according to published procedures. Recombinant Hpa1 was produced at the University of Manchester protein expression facility. The aptamer selection was performed in ELISA plates or Top yield PCR tubes, respectively, following immobilisation of the enzymes, application of the aptamer library, wash steps to remove non-binding species and elution using a step gradient from 300mM to 1.5M NaCl. Selected aptamer were cloned, sequenced and used in a variety of assays including

fluorescence quenching, ELISA, quartz crystal microbalance, fluorescence microscopy and enzyme inhibition assays, as well as immunohistochemistry.
Results: Aptamers were selected for both enzymes, and showed high affinity binding, in the low nanomolar range, and selectivity for their respective targets, as characterised by fluorescence quenching and ELISA. Enzyme inhibition assays demonstrated the capability of the aptamers to successfully inhibit their cognate enzymes, both in direct assays against the enzyme in the presence of its substrate, and in functional assays in tissue where the enzymes are naturally expressed, demonstrating their therapeutic potential. Fluorescent labelled aptamers were shown to successfully stain the enzyme in immunohistochemistry experiments, and were able to bind to the enzyme in quartz crystal microbalance assays, demonstrating the diagnostic potential of the aptamers. Following small modifications, these aptamers also demonstrated stability in serum and urine, exhibiting potential for *in vivo* use as inhibitors, or for analysis of biological material in diagnostic assays, including immunohistochemistry and as recognition units in biosensors.
Conclusions: Aptamers against tumour enzymes can prove a great alternative to antibodies and small molecule inhibitors, offering greater affinity, specificity and temperature stability, no immunogenicity, and great flexibility in a variety of modifications and uses.

Sunday 27 June 2010 14:35–16:05

Presidential Session
Presidential Session III

45 Use of somatically acquired genomic rearrangements as biomarkers in solid tumours

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Background: Somatically acquired genomic rearrangements are a feature of the majority of cancers and make attractive biomarkers for monitoring disease burden in cancer patients.
Material and Methods: Next-generation sequencing was used to rapidly identify genomic rearrangements in tumour samples obtained from three cancer patients (two with breast cancer and one with osteosarcoma). Somatically acquired rearrangements were confirmed by successful PCR and sequencing of the breakpoints in tumour but not matched normal genomic DNA samples. Highly specific quantitative PCR assays utilizing dual labeled probes were designed to a selected subset of rearrangements. Plasma or serum samples were obtained from each patient to investigate whether patient specific rearrangements could be detected. For one patient, serial serum samples were obtained in order to assess disease burden at multiple time points during treatment.
Results: Tumour-specific DNA rearrangements were successfully detected in plasma samples from each patient. PCR assays for rearrangements were able to detect a single copy of the tumour genome in many milliliters of plasma without false positives. Serial dilutions of templates indicate that results are quantitative through a 1000-fold dynamic range with amplification remaining in the linear range. Disease status, drug responsiveness and incipient relapse could all be monitored with a high degree of accuracy.
Conclusions: This proof-of-principle is the first concrete demonstration of the transforming power of whole cancer genome sequencing to personalise medicine in the oncology clinic. It is applicable across all types of solid tumours, adaptable to many therapeutic regimens and potentially capable of implementation within centralised molecular diagnostics laboratories.

46 An integrative structural and functional approach to pancreatic cancer gene discovery

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Background: Pancreatic cancer is among the most deadly of all cancers. Five year survival is less than 5%, and most patients succumb to the disease in three to six months after diagnosis. Despite its lethality, very few therapeutic options exist including virtually no targeted therapies. A better understanding of pancreatic cancer etiology is warranted.
Methods: Copy number alteration (CNA) was determined across seventy cell lines and early-passage xenografts using high-resolution Agilent 244K CGH arrays. Expression profiling was performed in parallel using Agilent catalog 44K arrays. Additionally, publicly available whole exome sequencing data were mined in order to identify genes showing both CNA and mutation. Identified candidate cancer genes were then simultaneously functionally interrogated using a pooled shRNA lentiviral library and carrying out a competitive growth screen across a panel of ten cell lines sampling the genetic diversity of pancreatic cancer.
Results: From the CGH, sequencing and expression data, we identified 147 genes with considerable structural evidence implicating them as candidate oncogenes or tumour suppressors. Among these, classical addictive oncogenes were identified by those shRNA hairpins depleted in the functional screen in cell lines harboring amplification and/or overexpression of the targeted candidate oncogene. Gatekeeper tumour suppressor genes were identified by those shRNA hairpins enriched in cell lines expressing target genes and also showing no phenotype where expression was already low. Positive control "known" cancer genes were included in all steps of analysis, and encouragingly this integrative approach to cancer gene discovery recovered nearly all of the well known pancreatic cancer genes. Additionally we have identified many novel candidate cancer genes which we are further characterizing.
Conclusions: We present here one of the richest compilations of structural data characterizing pancreatic cancer. We expand on our rich structural data to identify cell context specific oncogenes and tumour suppressors. This strategy naturally lends itself towards discovery of cancer genes ripe for targeted therapy, which is conspicuously absent from pancreatic cancer treatment regimens. In conclusion, we report an integrative structural and functional approach to identifying novel therapeutically attractive pancreatic cancer genes.

47 Molecular signatures of long non-coding RNAs in breast cancer patients

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Background: Of the ~3.3 billion bases of the human genome, only about 2% code for proteins. Since very recently, the remaining 98% have been considered to be 'junk' and functionless. However, large transcriptomic studies have shown that around 90% of the genome is actively transcribed of which a significant fraction may be functional and contribute to a previously underestimated regulatory layer of non-coding RNAs (ncRNAs). In several studies it has been shown that the expression of small ncRNAs, like miRNAs, is associated with diseases including cancer. However, the large group of long ncRNAs has drawn less attention despite their genome-wide distribution. These long ncRNA genes may contribute substantially to regulatory features ranging from epigenetic control and transcriptional regulation.
Material and Methods: For this pilot study we selected 25 breast carcinomas from a larger cohort of 920, representing the five clinically relevant tumour expression subclasses as well as normal breast tissue from breast reduction operations. Total RNA from these samples was analyzed utilizing the custom nONCOchip®, developed by the RNomics group at the Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany. The nONCOchip® covers both, experimentally identified cancer related nc RNAs of oncogenes (STAT-3), tumoursuppressor genes (p53), and cell cycle controlled genes, as well as known or predicted non-coding RNAs from public databases. The array includes in total 243,000 probes, with over 60,000 newly identified transcripts.
Results: The expression analyses of long non-coding RNAs showed that various long ncRNAs are expressed in breast tumours. Preliminary results using unsupervised clustering based on the non-coding RNA expression revealed groups of tumours with distinct expression patterns of long ncRNAs.
Conclusions: The growing list of ncRNA genes influencing carcinogenesis is striking. Long ncRNAs show distinct patterns of expression, divide the tumours in groups different from mRNA expression subgroups, and are likely to be involved in mRNA regulation structures. The identification of clinically relevant ncRNAs may open up for the development of new biomarkers and therapeutically tools that attack diseased cells.
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(*LOB and KR contributed equally to this study and should be regarded as joint first authors).