inconclusive association between region with greatest PD and subsequent tumour location (p = 0.07).
Conclusion: The findings help clarify the role of PD on breast cancer risk by suggesting that PD is a localized marked of risk.

37 Integration analysis between differentially expressed mRNA and miRNA induced by BRCA1 gene

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Background: Mutations within the BRCA1 tumour suppressor gene occur frequently in familial breast carcinomas and also decreased BRCA1 expression occurs in sporadic tumours. MicroRNAs (miRNA) are 20−25 nucleotide non-coding RNAs that inhibit the translation of targeted mRNA, and they have been implicated in the development of human malignancies, regulating a number of tumour suppressor genes (TSGs) and oncogenes. In our study we are to explore the relation between miRNA and the mechanisms of BRCA1 associated tumourigenesis.
Material and Methods: Whole genome transcriptional profiling covering >25,000 mRNA sequences and global miRNA expression profiling with >800 human miRNAs was performed on a Brca1 deficient, HCC1937 breast cancer cell line, and the isogenic HCC1937 expressing BRCA1. The miRNA targets were predicted with miRanda and TargetScan algorithms. Functional pathway enrichment was performed with the Ingenuity Pathway Analysis system.
Results: In our study we found over 8000 differentially expressed genes and 8 differentially expressed miRNAs between HCC1937 and HCC1937/BRCA1 cells (FDR $p < 0.05$). Subsequently, we integrated the mRNA and miRNA data to find statistically significant miRNA-mRNA relationships underlying the array signatures. Moreover, we identified a number of signaling pathways associated with these expression changes that included MAPK or NFκB pathway.
Conclusions: By this study we reveal the connection between miRNA, gene expression and pathways altered following expression of BRCA1 gene.

38 miR-449 induces apoptosis while triggering a stress and DNA damage response

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Background: The E2F1-responsive microRNA-34 family member miR-449 is a potent inducer of apoptosis[1], at least in part independently of p53. It displayed the highest expression in lung and trachea, while being strongly down-regulated in tumour cell lines, consistent with a tumour-suppressive activity. With this study, we aim to achieve a better understanding of the mechanisms leading to the induction of apoptosis, and to elucidate the role of miR-449 in normal cells.
Material and Methods: Using immunoblot analysis of H1299 cells (p53 −/−, non-small cell lung carcinoma) transfected with miR-449 or controls, we investigated the effects of miR-449 over-expression on potential target gene expression levels, on the DNA damage response, and on apoptosis-related pathways. The use of the caspase-inhibitor Z-VAD allowed us to discriminate between causes and consequences of caspase activation. In vitro cultivated aero-epithelial cells (AEC) were used to correlate the expression of miR-449 with the differentiation of bronchial epithelial cells.
Results: On top of inducing apoptosis and reducing CDK6 and SIRT1, miR-449 was able to accumulate gamma-H2AX, a common marker of DNA damage. The investigation of the DNA damage pathway revealed strong down-regulation of Chk1 and accumulation of phospho-p38-alpha. The siRNA knock-down of Chk1 alone was able to induce similar gamma-H2AX accumulation. Furthermore, strong up-regulation of miR-449 levels was observed upon differentiation of AEC.
Conclusions: Our results suggest that miR-449 leads to DNA damage accumulation through the down-regulation of an important cell cycle checkpoint, Chk1, thereby inducing apoptosis. Moreover, it may also target the Notch signaling linked protein DLL1, perhaps contributing to apoptosis or to bronchial epithelial differentiation, depending on the cellular context. E2F1-inducible microRNA 449a/b suppresses cell proliferation and promotes apoptosis.

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Presidential Session
Presidential Session II

39 Targeting the lactate transporter monocarboxylate transporter 1 constitutes a new therapeutic modality that disrupts a fundamental metabolic symbiosis in tumours

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Background: The glycolytic activity of hypoxic cells creates a gradient of lactate that mirrors the gradient of oxygen in tumours. In human tumours, high levels of lactate predict the likelihood of tumour recurrence, metastasis, and poor survival. In this study, we address the intrinsic contribution of the lactate anion to tumour growth and the tumour response to radiotherapy.
Materials and Methods: We initially selected SiHa and WiDr human cancer cell lines as metabolic archetypes of oxidative and glycolytic tumour cells, respectively. Metabolic profiling used enzymatic measurements and electron paramagnetic resonance oximetry. We used immunohistochemistry to detect the expression of the monocarboxylate transporter 1 (MCT1) in vitro and in vivo, including, under approval of the Duke University Institutional Review Board, in biopsies of human tumours. The significance of lactate uptake was tested by measuring intracellular pH, ATP level and cell survival, and by using the selective MCT1 inhibitor alpha-cyano-4-hydroxy-cinnamate (CHC) and specific siRNAs. The toxicity and therapeutic activity of CHC were tested in 3 different tumour models and mouse strains, with permission of local ethical boards. MCT1 inhibition and X-ray irradiation were used in combination to treat Lewis Lung carcinoma-bearing mice.
Results: We identified a metabolic symbiosis in tumours involving the recycling of lactate, released by glycolytic tumour cells, as an oxidative fuel for oxygenated tumour cells. The preferential use of lactate over glucose to fuel tumour cell respiration renders glucose available to fuel the glycolytic metabolism of hypoxic tumour cells. We further identified MCT1, selectively expressed at the plasma membrane of oxygenated tumour cells, as the prominent path for lactate uptake. We successfully disrupted the metabolic symbiosis by inhibiting MCT1 with a specific siRNA or with the selective inhibitor CHC, causing a switch from lactate-fueled respiration to glycolysis in oxygenated tumour cells. As a consequence, CHC delivery to tumour-bearing mice causes hypoxic/glycolytic tumour cell death by virtue of glucose starvation and the remaining oxygenated tumour cells were highly sensitive to radiotherapy. There was no overt toxicity. Validation of this new therapeutic strategy using and MCT1 expression in an array of primary human tumours provide clinical significance to anticancer MCT1 inhibition.
Conclusion: Tumours behave as metabolic symbionts that can be targeted therapeutically.
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40 TGFβ receptor inhibitors target the CD44high/Id1high glioma stem cell population in human glioblastoma

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Glioma is the most common tumour of the brain and its most aggressive form, called Glioblastoma multiforme (GBM), is one of the most aggressive and deadliest cancers with a median survival of around 14 months. In the last years a cell subpopulation have been described in GBM, the Glioma Stem Cells (GSCs), also called glioma-initiating cells. GSCs have characteristics similar to normal stem cells and are responsible for tumour initiation, relapse and therapeutic resistance. GSCs should then be considered critical therapeutic targets. In addition, it has been showed that TGFb signaling pathway has an important role in cancer. In particular, in high grade glioma, TGFb acts as an oncogenic factor.
Here, we show that TGFβ inhibitors, currently under clinical development, target the GSC compartment in human GBM patients. Using patient-derived specimens, we have determined the gene signature of TGFβ inhibition in human GBM which includes Id1 (inhibitor of differentiation 1) and Id3 transcription factors. Id1 has been shown to be expressed in B1 type adult neural stem cells where it has an important role in the regulation of the self-renewal capacity. More importantly, in cancer Id1 has been shown to be expressed in tumours and described to be involved in metastasis.
We have identified a cell population enriched for GSCs that is characterized by the expression of high levels of CD44 and Id1. The inhibition of the TGFβ pathway decreases the $CD44^{high}/Id1^{high}$ GSC population through the

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