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

L. Prokunina-Olsson[1], Y.P. Fu[1], H. Edvardsen[2], A. Kaushiva[1], I. Kohaar[1], P. Porter-Gill[1], S. Fosså[3], B. Naume[4], A.L. Børresen-Dale[2], V. Nedelcheva Kristensen[2]. *[1]National Cancer Institute, Laboratory of Translational Genomics, Bethesda MD, USA, [2]Institute for Cancer Research Oslo University Hospital, Department of Genetics, Oslo, Norway, [3]The Norwegian Radium Hospital University of Oslo, Faculty Division of Medicine, Oslo, Norway, [4]Oslo University Hospital Radiumhospitalet, Department of Oncology, Oslo, Norway*

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

O. Cuvillier[1], I. Ader[1], P. Bouquerel[1], M. Golzio[1], B. Malavaud[1], R.A. Sabbadini[2]. *[1]Institute of Pharmacology and Structural Biology, Cancer Biology Department, Toulouse, France, [2]LPath Inc., San Diego, USA*

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

C.E. Ford[1], J. Tuomela[2], E.L. Hedditch[1], L. Axelsson[3], Q. Liu[3], R.L. Ward[1], P. Härkönen[2], T. Andersson[3]. *[1]University of New South Wales, Adult Cancer Program Lowy Cancer Research Centre, Sydney, Australia, [2]University of Turku, Cell Biology & Anatomy, Turku, Finland, [3]Lund University, Experimental Pathology, Malmö, Sweden*

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.

Reference(s)

[1] Jönsson M, et al. Cancer Res 2002; 62: 409−16.
[2] Ford CE, et al. Proc Natl Acad Sci USA 2009; 106: 3919−24.
[3] Säfholm A, et al J Biol Chem. 2006; 281: 2740−9.

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

S. Missailidis[1], S. Arnold[1], S.C. Simmons[1], M. Velasco-Garcia[1], E.A. McKenzie[2], K. Kantiotou[3], G. Sotiropoulou[3]. *[1]The Open University, Chemistry and Analytical Sciences, Milton Keynes, United Kingdom, [2]University of Manchester, Life Sciences, Manchester, United Kingdom, [3]University of Patras, Pharmacy, Patras, Greece*

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