

Material and Methods: The individual PI3K isoforms were targeted by either siRNA or isoform-specific pharmacological inhibitors. Additionally, combinational treatments with isoform-specific inhibitors and chemotherapeutic agents were performed. Cell proliferation (MTS assay), colony formation (soft agar assay), and induction of apoptosis (caspase activity by Caspase Glo 3/7 assay and PARP cleavage by WB) were measured after treatment. Activation of the PI3K/Akt signaling pathway was analyzed by using phosphorylation site-specific antibodies of downstream elements (western blot).

Results: Concentration dependent decrease in GBM cell proliferation was observed when using two distinct p110 α -specific inhibitors. Targeting p110 α additionally impaired anchorage-independent growth and down-regulation of the isoforms p110 α or p110 β by siRNA induced apoptosis in GBM cells. In agreement with these observations, treatment of GBM cells with p110 α -specific inhibitors led to decreased activation of Akt and phosphorylation of the ribosomal protein S6. Combinational treatment of p110 α inhibitors with chemotherapeutic agents led to a sensitization of GBM cells to chemotherapy.

Conclusions: Targeting individual PI3K isoforms has an impact on cellular responses, though it might be necessary to target more than one isoform simultaneously. This study will lead to a better understanding of the specific functions of class I α PI3K isoforms in human GBM cell biology and will thus help in developing new targeted therapies to cure this common type of malignant brain tumour.

[360] Role of DLL4 and JAG1 in tumour angiogenesis

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Background: The notch signalling pathway plays a crucial role in vascular development and physiology. Delta-like 4 (DLL4) and Jagged1 (JAG1) are two key notch ligands that are implicated in angiogenesis. The aim of this work is to study the functional difference between these two ligands *in vitro* and *in vivo*.

Material and Methods: HUVECs were seeded on DLL4 or JAG1 coated plates in a time series experiment and subjected to quantitative PCR to study the regulation of notch target genes such as HEY1, HEY2, EFNB2 and DLL4. Two U87 cell lines were generated which over-expressed murine DLL4 or murine JAG1. Growth of these cell lines *in vitro* or as subcutaneous tumours *in vivo* was compared to that of control wild-type U87 cell line. Subsequent changes in vascular phenotype, markers of hypoxia (CA9), proliferation (MIB-1) or apoptosis (TUNEL), and notch downstream target genes as mentioned above were then assessed.

Results: Preliminary quantitative PCR results showed that JAG1 is less potent than DLL4 in stimulation of notch target genes in HUVECs. The growth of U87 cell lines which over-expressed murine DLL4 or murine JAG1 was slower compared to wild-type U87 cell line *in vitro*. This inhibitory effect was not observed *in vivo*, where the cell lines over-expressing mDLL4 or mJAG1 grew faster than the wild type control cell line as subcutaneous tumours ($P < 0.01$). The two over-expressing notch ligand cell lines displayed the phenotype of lower number of apoptotic and proliferative cells compared to control tumours ($P < 0.05$) while U87 mDLL4 displayed a lower magnitude of hypoxia compared to control tumours ($P < 0.01$). Interestingly, tumours over-expressing mDLL4 had larger vessels compared to control, whereas mJAG1 produced more yet functional vessels ($P < 0.01$); both cell lines significantly reduced pericyte coverage ($P < 0.01$).

Conclusions: In conclusion, JAG1- and DLL4-notch signalling have different effects on vessel formation, which impacted on the growth of the tumours *in vivo*. The mechanism behind the differential responsiveness of the notch receptors to DLL4 or JAG1 ligands could either reflect modulation by fringes, a family of glycosyltransferases that regulate notch signalling or by a positive feedback loop present for DLL4-notch signalling only. This work has highlighted some key novel differences between the two notch ligands, which require further investigation and is highly relevant for anti-angiogenic therapy.

[361] Targeting phosphoinositide 3-kinase signalling in small cell lung cancer

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The phosphoinositide 3-kinase (PI3K) pathway, fundamental for cell proliferation, survival, and motility, is known to be frequently altered and activated in neoplasia, including carcinomas of the lung. Based on the high frequency of alterations, targeting components of the PI3K signalling pathway is considered to be a promising therapeutic approach in cancer treatment. In

this study we investigated the potential of targeting PI3K signalling in small cell lung cancer (SCLC), which is the most aggressive of all lung cancer types and almost entirely related to smoking. SCLC accounts approximately 15–20% of all lung cancer cases. Although most patients initially respond to chemotherapy, the overall 5-year survival is less than 5%. In order to control metastasis and chemoresistance, new targets for development of drug therapies are urgently required. An immunohistochemistry analysis in primary SCLC tissue samples showed over-expression of the PI3K isoforms p110 α and p110 β , compared to normal lung tissue. By targeting the PI3K isoforms p110 α or p110 β with specific pharmacological inhibitors or small interfering RNA (siRNA), we could observe strongly affected cell viability in SCLC cells. Expression and phosphorylation state of PI3Ks and signalling molecules were studied by Western blot and Taqman analysis. Downstream signalling molecules like vascular endothelial growth factor A (VEGFA) showed a downregulated gene expression in response to PI3K inhibition. We could also observe decreased phosphorylation levels in PI3K pathway components, such as Akt, ribosomal S6 protein, and 4E-BP1, and reduced expression of cell cycle-related molecules, like p21, Cyclin D₁, and Cyclin E. A DNA microarray analysis was performed in H69 SCLC cells treated with the inhibitors PIK75 (p110 α) or TGX221 (p110 β). In particular, PIK75 strongly affected gene expression in widespread functional categories. As targets for further investigation we chose genes related to cell growth, proliferation, cell cycle, and cell survival. A phosphorylation antibody array performed in H69 cells showed that targeting p110 α with PIK75 led to a decreased activation state of PI3K/Akt/mTOR pathway components. Together, our studies will lead to a better understanding of the biological function of PI3K isoforms in controlling cell responses, such as proliferation, apoptosis, and metastasis in SCLC. Furthermore, this knowledge could point out novel targets and contribute to the development of urgently required new therapies

[362] Targeting the glycolytic cancer cell – cancer-specific modulation of NAD⁺ and NADH and implications for anti-cancer therapy

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Cancer and non-cancer cells differ fundamentally in their metabolism and this may be exploited therapeutically. Cancer cells are metabolically reprogrammed to utilise glycolysis as their primary source of energy even in the presence of oxygen. To fuel glycolysis cancer cells avidly consume NAD⁺. Modulation of NAD⁺ levels by inhibiting enzymes involved in NAD⁺ biosynthesis is a recognised strategy for targeting cancer's "sweet tooth". However clinical trials indicate damage to normal tissues by this approach consistent with non-cancer cells requiring a basal level of NAD⁺ for normal cell functions. We demonstrate by RNAi that by targeting a key enzyme involved in the glycolytic switch (lactate dehydrogenase A) it is possible to selectively modulate the NADH/NAD⁺ redox status of cancer cells and not that of non-cancer cells. We also show that: (i) this may enable cancer-selective targeting of NAD(H)-dependent enzymes with critical roles in cancer survival but for which direct targeting is problematic because of functions in non-cancer cells (eg. SIRT1); and (ii) this can increase the efficacy of redox-sensitive anti-cancer drugs (eg. EO9) in a cancer-selective manner. These findings identify a unique strategy for selectively targeting cancer and increasing the effectiveness of certain current anti-cancer drugs (via combinatorial therapy) based upon exploiting a fundamental metabolic difference between cancer and non-cancer cells.

[363] The secretory small GTPase Rab27B drives poor prognosis in ER-positive breast cancer

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Background: Vesicle exocytosis, controlled by secretory GTPases such as Rab27B, delivers critical pro-invasive growth regulators into the tumour microenvironment. The biological role and expression status of Rab27B in breast cancer was unknown.

Materials and Methods: Rab27B was studied in estrogen receptor (ER)-positive human breast cancer cell lines (MCF-7, T47D, ZR75.1) using GFP-fusion constructs, including wild type Rab3D, Rab27A, Rab27B and Rab27B point mutants defective in GTP-binding or geranylgeranylation, and transient siRNA targeting. In cell culture, cell-cycle progression was evaluated by flow cytometry, Western blotting and measurement of cell proliferation rates, invasion was assessed using Matrigel and native collagen type I substrates. Orthotopic tumour growth, local invasion and metastasis were analyzed in mouse xenograft models. Mass spectrometry was performed to identify Rab27B-secreted pro-invasive growth regulators. In clinical breast cancer, Rab3D, Rab27A and Rab27B mRNA levels were analyzed by quantitative RT-PCR (n=20) and Rab27B protein level was evaluated by immunohistochemistry (n=60). Statistical tests were two-sided.