it significantly enhanced the inhibition of sprout angiogenesis by echistatin. These data suggest that collagen and fibrin differentially, but synergistically regulate sprout angiogenesis.

356 The putative cannabinoid receptor GPR55 participates in the control of cancer cell proliferation

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Background: Cannabinoids, the active components of marijuana and their derivatives, induce a biphasic effect on cancer cell proliferation: while "low" concentrations increase cell proliferation, "high" concentrations exert an antiproliferative action. Two cannabinoid receptors, CB_1 and CB_2, have been cloned so far. Recently, the orphan receptor GPR55 has been proposed as a new cannabinoid receptor. In this context, we analyzed the involvement of GPR55 in cannabinoid-induced modulation of cancer cell proliferation.
Material and Methods: We studied the expression of GPR55 in 24 human cancer cell lines and 47 breast tumours by real-time quantitative PCR, and in 37 breast tumours, 157 gliomas and 19 pancreatic cancers by analyzing previously published microarray databases. We also modulated the expression of GPR55 in several human cancer cell lines with selective siRNA or overexpression vectors, and analyzed the proliferative response of the cells to Δ^9-tetrahydrocannabinol (THC, the main cannabinoid in marijuana) by the MTT test. The involvement of ERK-MAPK in THC-induced GPR55-mediated effect on cancer cell proliferation was assesed by Western blotting and by pharmacological blockade of this cascade with the MEK inhibitor U-0126.
Results: We observed that most human cancer cells express GPR55 and found a correlation between GPR55 expression and histological grade in human gliomas, breast tumours and pancreatic tumours. Furthermore, we observed that glioma patients with higher GPR55 levels have decreased survival rates.
Our cell culture experiments show that GPR55 knockdown abolishes the pro-proliferative response of cancer cells to low THC concentrations, while GPR55 overexpression had the opposite effect (enhancement of cell proliferation). U-0126 was able to block THC proliferative action in cells ectopically overexpressing or endogenously expressing GPR55.
Conclusions: Our results indicate that GPR55 could be a marker of tumour aggressiveness (high histological grades, poor differentiation, low survival rates) and that this receptor mediates part of the well known effects of cannabinoids on cancer cell proliferation via ERK modulation. In summary, evidence presented here introduces the GPR55 receptor as a new potential target for the management of cancer.

357 Characterization of the transcriptional networks involving PEA3 transcription factors during mammary morphogenesis and tumourigenesis

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Background: PEA3 transcription factors (Pea3, Erm and Er81) belong to the ETS family. They are functionally linked to epithelial branching morphogenesis in organs like kidney or mammary gland and a deregulation of their expression is often associated with cancer progression and aggressiveness. As transcription factors, PEA3 proteins modulate specific target genes expression. Therefore, we aim at understanding the molecular mechanisms involving the PEA3 proteins during mammary gland morphogenesis and tumourigenesis by defining and studying their target genes.
Material and Methods: We use two cell lines as models of mammary morphogenesis (normal mammary epithelial cells) or tumourigenesis (tumourigenic mammary epithelial cells). In these models PEA3 transcription factors expression is modulated by siRNA mediated knockdown or stable overexpression. Cells are then used for *in vitro* and *in vivo* phenotypic assays (migration, invasion, proliferation, tumour formation). The definition of the target genes is made with a global transcriptomic approach based on microarray technology.
Results: We showed that PEA3 proteins Pea3 and Erm are able to drive mammary epithelial cells morphogenesis within a collagen matrix and to take part in mammary tumourigenesis. More precisely, these effects are associated with a modulation of the migration and invasion abilities. A large scale comparative analysis of both cell lines transcriptome allowed us to decipher the transcriptional networks controlled by PEA3. Thus, we showed that most of these newly identified PEA3 potential target genes are already known modulators of cell proliferation, migration and invasion.
Amongst these we focused on the *cyclinD2* gene. It encodes two isoforms generated through an alternative splicing event. We show that PEA3 differentially regulates the expression of both isoforms. In accordance, mammary epithelial cells stably expressing cyclinD2 isoforms show different morphogenetic abilities. Finally, using an siRNA isoform specific knockdown we test the relationship between PEA3 and the cyclinD2 isoforms in a context of PEA3 induced *in vitro* morphogenesis.
Conclusion: This study allows to understand the molecular mechanisms involving PEA3 proteins during the events leading to mammary morphogenesis and tumourigenesis. We identified new potential target genes and are now defining their precise role during PEA3 induced morphogenesis and tumourigenesis. This strategy should help in defining new therapeutic markers or targets for the treatment of breast cancer.

358 Oncogenic function of smoothened in T-cell lymphoblastic lymphomas

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Background: The activation of GLI/Hedgehog pathway has been related to normal processes of cellular differentiation, as well as to the development of numerous oncogenic processes. In this work we show how this pathway is abnormally activated on murine T-cell lymphoblastic lymphomas induced by γ-irradiation.
Material and Methods:
- Induction of Lymphoblastic Lymphomas T by gamma radiation on susceptible strain mice (C57BL/6J).
- Isolation of RNA, DNA and protein from thymus, thymic cell fraction and stroma-enriched cell fraction were obtain by TriPure™ (Roche) protocol.
- Quantification of the transcriptional levels of Smoothened gene was performed by real-time quantitative RT-PCR with a LightCycler instrument (Roche). RT-PCR reactions were carried out in total RNA using the one-step LightCycler SYBR Green I kit (Roche).
- Quantification of the Smoothened protein levels was performed through Western Blot using a Smoothened Drosophila Homolog (SMO) anti-Mouse anti-Human Polyclonal Antibody from MBL international corporation.
- Quantification of apoptosis by TUNEL assay was performed using a commercially available kit (Roche) and measuring the percentage of TUNEL positive cells on a FACSCalibur flow cytometer.
- Quantification of Cell Cycle assay was done through Propidium Iodide staining on 70% ethanol-fixed cells, and measuring the results on a FACSCalibur flow cytometer.
- Luciferase assay was done using *Dual-Luciferase Reporter Assay System* (Promega).

Results: Using genomic analysis by cDNA-arrays we demonstrated the overexpression of the gene *Smoothened* – the only non-redundant component in this pathway-in this type of lymphomas. Then, we validated this result analyzing *Smo*-RNA levels by real time quantitative RT-PCR and Smo-protein levels by western-blot. Despite overexpression can be detected in the tumoural thymocytes, our results evidenced how the isolated thymic stroma fraction is which exhibits the highest levels of expression. The overexpression of *Smo* was confirmed on human cell lines derived from T-cell lymphoblastic leukemia/lymphomas, and also on primary human lymphoblastic lymphomas. Furthermore, the transfection with luciferase vectors carrying specific binding sites for Gli transcription factors allowed us to confirm that the overexpression of Smo leads to the activation of the Gli/Hh pathway. The effects caused by Smo over-expression were confirmed using Cyclopamine – a Smo specific inhibitor.
Conclusions: Our results show that Smoothened has an oncogenic function in T-cell lymphoblastic lymphomas.

359 Targeting class IA phosphoinositide 3-kinase isoforms in glioblastoma

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Background: Glioblastoma (GBM) is the most common tumour of the central nervous system and it is characterized by a highly invasive phenotype, resistance to chemotherapy and radiotherapy, as well as poor patient survival chances. Phosphoinositide 3-kinases (PI3K), a class of lipid and protein kinases, play an important role in intracellular signaling. Furthermore, the pro-survival PI3K/Akt signaling pathway is often deregulated in cancer. The most prominent deregulations include mutations and/or deletions of the tumour suppressor gene phosphatase and tensin homologue (*PTEN*) and activating mutations in the oncogene *PIK3CA* (encoding class I_A p110α). This study further investigates the role of class I_A PI3K isoforms (p110α, p110β, and p110δ) in respect to signaling pathway activation, cell proliferation, and resistance to chemotherapeutic agents in human GBM cell lines and *ex vivo* cultures.

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 p110α-specific inhibitors. Targeting p110α additionally impaired anchorage-independent growth and down-regulation of the isoforms p110α or p110β by siRNA induced apoptosis in GBM cells. In agreement with these observations, treatment of GBM cells with p110α-specific inhibitors led to decreased activation of Akt and phosphorylation of the ribosomal protein S6. Combinational treatment of p110α 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_A 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 p110α and p110β, compared to normal lung tissue. By targeting the PI3K isoforms p110α or p110β 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 D_1, and Cyclin E. A DNA microarray analysis was performed in H69 SCLC cells treated with the inhibitors PIK75 (p110α) or TGX221 (p110β). 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 p110α 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.