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

C. Andradas[1], M.M. Caffarel[2], M. Salazar[1], E. Pérez-Gómez[1], M. Lorente[1], G. Velasco[1], M. Guzmán[1], C. Sánchez[1]. [1]*Universidad Complutense de Madrid, Biochemistry and Molecular Biology I, Madrid, Spain,* [2]*University of Cambridge, Pathology, Cambridge, United Kingdom*

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

F. Ladam[1], H. Leger[2], Z. Kherrouche[1], D. Tulasne[1], A. Benecke[2], Y. de Launoit[1], A. Chotteau-Lelièvre[1]. [1]*Institut de Biologie de Lille, UMR8161 CNRS, Lille, France,* [2]*Institut de Recherche Interdisciplinaire, Systems Epigenetics Group, Lille, France*

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

H. Gonzalez Gugel[1], M. Villa-Morales[1], J. Fernández-Piqueras[1]. [1]*Centro de Biología Molecular "Severo Ochoa" (CBMSO), Cellular Biology and Immunology, Madrid, Spain*

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

K. Höland[1], D. Boller[2], K. Frei[3], A. Arcaro[1]. [1]*University of Bern, Department of Clinical Research, Bern, Switzerland,* [2]*University Children's Hospital Zurich, Department of Oncology, Zurich, Switzerland,* [3]*University Hospital Zurich, Department of Neurosurgery, Zurich, Switzerland*

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.