c-Fos functions in epidermal development, homeostasis and tumourigenesis are not yet fully understood.
Material and Methods: Gain of function studies are performed using an inducible, epithelial-specific transgenic mouse model for ectopic *c-fos* expression. The carcinogen 7,12-dimethylbenz[α]anthracene (DMBA), inducing activating Ras mutations, is used as a tumour initiator. Protein expression is evaluated by immunohistochemistry using frozen and paraffin-sections and by immunoblotting. RNA expression analyses are performed using qRT-PCR and cytokine levels are measured by ELISA.
Results: Ectopic epidermal-specific *c-fos* expression in adult mice induces epidermal hyperplasia. Moreover, *c-fos* expression in combination with the carcinogen DMBA, is sufficient to promote the formation of highly invasive Squamous Cell Carcinomas (SCC) of the Achantolytic subtype. We also demonstrate the presence of an immune cell infiltrate mainly composed of $CD4^+$ and $CD8^+$ T lymphocytes as well as $F4/80^+$ macrophages, both in the hyperplastic skin and in the stroma of the SCCs. Interestingly, serum levels of IL-6 are increased both in transgenic c-Fos DMBA-free or DMBA-treated mice. Finally, immunohistochemical analyses indicate that human SCCs express high levels of c-Fos.
Conclusions: These results show that c-Fos can induce epidermal hyperplasia and that in combination with DMBA, it is sufficient for the development of SCCs. Expression of c-Fos in human SCCs suggests that inhibition of Fos/AP-1 might be a viable therapeutic option.

351 Identification and analysis of two novel Mdm2-interacting proteins involved in the regulation of cellular stress response pathways

K. Cetkovska[1], S. Uldrijan[1], K.H. Vousden[2]. [1]*Masaryk University Faculty of Medicine, Department of Biology, Brno, Czech Republic,* [2]*Beatson Institute for Cancer Research, Tumour Suppression Laboratory, Glasgow, United Kingdom*

Background: Tumour suppressor p53 is a key regulator of cellular responses to stress stimuli such as DNA damage, ribosomal stress or hypoxia. Oncoprotein Mdm2, which is often found overexpressed in cancers, serves as a ubiquitin ligase for p53 and promotes p53 degradation via 26S proteasome. In addition to the N-terminal p53-binding domain and the C-terminal RING finger domain, the Mdm2 protein contains a centrally located domain rich in acidic amino acids whose function hasnt been fully explained yet. It has been shown that this part of the Mdm2 protein is required for efficient ubiquitylation and degradation of p53. Another important role of the central region of Mdm2 is the binding of numerous Mdm2 regulators such as YY1, p300, p14Arf, etc. The aim of our project is to identify and characterize new Mdm2-interacting proteins that might regulate Mdm2 function in tumour cells by binding to the central part of Mdm2 oncoprotein.
Methods and Material: To identify novel binding partners for Mdm2 we used tandem affinity tag purification of cellular complexes containing Mdm2, followed by mass spectrometry analysis. Co-immunoprecipitations and immunofluorescence were used to confirm the interactions between Mdm2 and selected candidate proteins. The function of the binding partners was further analysed in various functional assays (e.g. degradation, ubiquitylation, and promoter activity assays) in human cancer cell lines.
Results: We have identified basal transcription factor TFII-I and ubiqutin-specific protease USP48 as new binding partners for Mdm2. Our data indicate that TFII-I promotes Mdm2-mediated p53 ubiquitylation and could be also involved in the regulation of Mdm2 protein levels. On the other hand, TFII-I has been implicated in cellular responses to certain types of the stress and we show that Mdm2 can inhibits the transcriptional activity of TFII-I, suggesting that Mdm2 might take part in the regulation of TFII-I-mediated stress responses.
Our results show that the second identified Mdm2-interacting partner USP48 can also modulate the levels of p53 ubiquitylation. In addition to that, USP48 is able to stabilize Mdm2. However, our data suggest that rather than simply catalyzing Mdm2 deubiquitylation, the role of USP48 in the regulation of Mdm2 protein levels could be more complicated.
Conclusion: We have identified two novel Mdm2-interacting proteins, general transcriptional factor TFII-I and ubiqutin-specific protease USP48. Both proteins can regulate the extent of tumour suppressor p53 ubiquitylation and the cellular levels of oncoprotein Mdm2. In addition to that, the overexpression of Mdm2 found in some types of cancer could influence TFII-I-directed responses to certain stress stimuli.
This work was supported by Cancer Research UK and the Czech Science Foundation grant No. 301/09/1324.

352 Withdrawn

353 ALK kinase controls an angiogenetic program in lymphoma, lung carcinoma and neuroblastoma

C. Martinengo[1], C. Mastini[1], M. Menotti[1], C. Ambrogio[1], E. Pellegrino[1], R. Piva[1], P. Perri[2], M. Ponzoni[2], C. Voena[1], R. Chiarle[1]. [1]*University of Torino, Department of Biomedical Science and Human Oncology, Torino, Italy,* [2]*G. Gaslini Children's Hospital, Experimental Therapy Unit Laboratory of Oncology, Genova, Italy*

Background: A portion of haematological and solid cancers, such as Anaplastic Large Cell Lymphoma (ALCL), Non Small Cell Lung Carcinoma (NSCLC) and neuroblastoma (NB), may express constitutive active forms of the Anaplastic Lymphoma Kinase (ALK). Constitutively active ALK, mainly in the form of translocations or point mutations, acts as an oncogene in lymphomas and, potentially, in NSCLC and NB. These cancer need to activate an angiogenetic program to sustain their growth. Thus, we investigated the relation between oncogenic ALK and angiogenesis in these cancers.
Material and Methods: We investigated the existence of an ALK-induced angiogenetic program in ALK transformed cells, in particular the expression of VEGF, Hif-1α and Hif-2α. ALCL cells lines TS and SU-DHL1 that carry an NPM-ALK translocation, H2228 and H3122 NSCLC that carry an EML4-ALK translocation, SH-SY5Y NB that have an ALK activating point mutation were used in the experiments. ALK inhibitors or shRNA specific for ALK or ALK-directed siRNA entrapped into liposomes were used to block ALK kinase activity. Gene expression profiling, microarrays, Western Blots were performed on ALCL, NSCLC and NB cells incubated in normoxia, hypoxia (3% O_2), or treated with deferoxamine (DFX), an hypoxia-mimetic compound. Xenografts in immunodeficient mice from ALK positive ALCL lines were treated with bevacizumab.
Result: In ALCL cell lines incubated in normoxia, the inhibition of ALK tyrosine kinase activity significantly decreased VEGF secretion. In ALCL cells incubated in hypoxia or with DFX, ALK inhibition lead to a dramatic reduction of Hif-2α mRNA and protein levels, whereas Hif-1α was less affected. Comparable reduction of Hif-2α after ALK inhibition were observed in H3122 and in SH-SY5Y cell lines. In ALCL, a specific shRNA against Stat3, a pivotal mediator of ALK transforming activity, induced a decrease of Hif-2α protein levels. Finally, treatment with bevacizumab of xenografts lead to a significant delay in ALCL growth. In a subcutaneous mouse model of NB, intratumoural injection of NB-targeted ALK-siRNA liposomes inhibited blood vessel density.
Conclusion: The tyrosine kinase ALK controls a common, Stat3-mediated angiogenetic program in ALCL, NSCLC and NB cells by regulating secretion of VEGF and Hif-2α protein levels. Inhibition of ALK activity reduces angiogenesis in ALK positive cancers and treatment of ALK tumours with anti-angiogenetic drugs is beneficial in reducing tumour growth.

354 Withdrawn

355 Fibrin and type I collagen 3D matrix differentially regulate sprout angiogenesis of human dermal microvascular endothelial cells

X. Feng[1], M. Shaker[2], R. Clark[3], M. Tonnesen[4]. [1]*California Northstate College of Pharmacy, Pharmaceutics and Biomedical Sciences, Rancho Cordova, USA,* [2]*Albany College of Pharmacy, Pharmaceutical Research Institute, Albany, USA,* [3]*State University of New York at Stony Brook, Dermatology Department of Medical School, Stony Brook, USA,* [4]*Veterans Affairs Medical Center, Dermatology Section, Northport, USA*

Angiogenesis is a highly regulated event involves complex, dynamic interactions between microvascular endothelial cells and extracellular matrix (ECM) proteins. Alteration of ECM composition and architecture is a hallmark of wound clot and tumour stroma. However, the role of ECM in regulation of angiogenesis associated with wound healing and tumour growth is not well defined. During angiogenesis, endothelial cell responses to growth factors are modulated by the compositional and mechanical properties of a surrounding three-dimensional (3D) extracellular matrix (ECM) that is dominated by either cross-linked fibrin or type I collagen. In this study, we investigated the correlation of sprout angiogenesis and ECM environment using in vivo and in vitro angiogenesis models. In healing full-thickness cutaneous porcine wounds, the fibrin-rich early granulation tissue in 5 day wounds was filled with newly formed vessels. Then the angiogenic neovessels in early granulation tissue mature and then regress as fibrin was replaced by collagen in the wound space. It suggests that provisional matrix, especially fibrin, is essential for sprout angiogenesis. Using an in vitro 3D microcarrier based sprout angiogenesis system we further demonstrated that fibrin and type I collagen 3-D matrix differentially regulated angiogenic sprout formation of human dermal microvascular endothelial cells (HDEMC). Expression of integrin αvβ3 is one of the hallmark features of sprout angiogenesis. Remarkably, integrin β3 expression was highly up-regulated in vasular endothelial cells found in fibrin rich, but not in collagen rich matrix environment in vivo and in vitro. Echistatin, a disintegrin specific for αvβ3, dose dependently inhibited sprout angiogenesis of HDMEC in fibrin. While blocking antibody to integrin α2β1, receptor for collagen, had no inhibitory effect on sprout angiogenesis in vitro,

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.