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

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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.
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352 Withdrawn

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

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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

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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,