be co-grafted with other cell types to explore intercellular communication during vessel formation. Co-implantation of HUVEC with pericytes led to an increase in vessel density and perfusion. α-SMA and Desmin coverage were also increased. Fourth, implantation of lymphatic endothelial cells allows the investigation of the lymphangiogenic potential of different LEC populations and the mechanism of LEC differentiation. Implantation of LEC spheroids with normal human dermal fibroblasts led to the formation of podoplanin- and CD31-positive vascular structures. Electron microscopy revealed typical lymphatic structures with intercellular gaps and numerous endocytotic vesicles. In conclusion, the EC spheroid transplantation assay offers numerous applications for the field of vascular research and beyond. The simplicity and robustness of the assay promise to make it a versatile tool to study EC functions and to improve the development of anti-angiogenic therapies.

383 Trapping and silencing of npm-alk in the nucleus is a fundamental event for npm-alk mediated cell transformation

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Background. Anaplastic Large Cell Lymphoma (ALCL) is a subtype of Non Hodgkin's lymphoma characterized by translocations of the Anaplastic Lymphoma Kinase (ALK) gene with a variable partner gene, most frequently Nucleophosmin (NPM1). ALK fusions have oncogenic tyrosine kinase activity. Different ALK chimeras localize to distinct cellular compartments. The NPM-ALK protein is the only ALK chimera localized both in the cytoplasm and in the nucleus, but the role of NPM-ALK cytoplasmic or nuclear fractions on cellular transformation is undetermined.
Material and Methods: NPM-ALK fusion is composed of the NPM N-terminal and ALK the C-terminal. We generated a construct with the entire NPM coding sequence fused to the C-terminal of ALK (NPMtot-ALK) in order to force NPM-ALK expression in the nucleus. Next, NPMtot-ALK was fused to ER binding domain (NPMtot-ALK-ER) in order to redirect its localization in the cytoplasm. All constructs were expressed in 293T, NIH3T3, IL3-dependent BAF/3 cells and in MEFs double KO for NPM and p53 ($NPM^{-/-}/p53^{-/-}$). Immunohistochemistry, immunofluorescence and WB were performed on these cells. Transformation properties of the different constructs were tested *in vitro* by soft-agar assays. Apoptosis was measured by TMRM staining.
Results: In 293T and NIH3T3 cells, NPM-ALK and the kinase dead $NPM\text{-}ALK^{K210R}$ were localized both in the nucleus and in the cytoplasm, NPMtot-ALK was localized in the nucleus, whereas NPMtot-ALK-ER only in the cytoplasm. In NIH3T3 cells, NPMtot-ALK nuclear localization led to its dephosphorylation and abrogation of the downstream signalling. Moreover, NPMtot-ALK was not able to transform NIH3T3 fibroblasts in soft agar assays. NPMtot-ALK-ER was mainly cytoplasmatic and caused apoptosis. In addition, BAF/3 cells ectopically expressing NPM-ALK were able to grow in absence of IL3, while BAF/3 expressing $NPM\text{-}ALK^{K210R}$ or NPMtot-ALK were not. Finally, in $NPM^{-/-}/p53^{-/-}$ MEFs, NPM-ALK was localized only in the cytoplasm and resulted in cell death.
Conclusions: The nuclear portion of NPM-ALK is not phosphorylated and not capable of transforming cells. However, redirection of NPM-ALK entirely to the cytoplasm causes cell death. WT NPM dimerizes with a fraction of NPM-ALK fusion and entraps it in the nucleus in an inactive form. Thus, the ratio between nuclear and cytoplasmic localization is critical for NPM-ALK phosphorylation, signalling and transforming capability.

384 Dicer1 is a synthetic lethal partner of tumour suppressor p53

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Background: A large body of evidence indicates that alterations in the expression of miRNAs contribute to cancer pathologies [1]. *DICER1*, which encodes an enzyme playing a critical role in miRNA biogenesis, is the target of mutations or haploid deletions in human cancers [2,3]. We have recently shown that *Dicer1* is a haploinsufficient tumour suppressor in mice [4]. Surprisingly, however, homozygous deletions or loss-of-function mutations in *DICER1* have never been found in human tumours and homozygous loss of *Dicer1* is strongly selected against in a K-Ras-induced mouse model of lung cancer [3]. These observations raise the possibility that Dicer1 is required for tumour formation.
Materials and Methods: *Mice: Chx10-cre,* $Rb^{lox/lox}$, $p107^{-/-}$, $p53^{lox/lox}$ mice were intercrossed with $Dic^{lox/lox}$ mice.
Immunohistochemistry: Eyes were fixed overnight in 4% paraformaldehyde/PBS, and paraffin embedded. 5 μm sections were immunostained with the different antibodies.
microRNA expression analysis: Total RNA was prepared from dissected retinea or isolated tumours using the miRNeasy kit (Qiagen) according to the manufacturer's instructions. Profiling of these samples was performed as described previously [5, 6].
Results: We show that complete ablation of *Dicer1* in retinal progenitor cells prevents the formation of retinoblastoma in mice that harbour inactivating mutations targeting both the Rb and p53 tumour suppressor pathways. Importantly, loss of *Dicer1* in retinal progenitor cells does not affect survival (nor retinogenesis) in the absence of Rb, however, it induces their apoptotic death upon concomitant inactivation of p53. miRNA profiling of Rb/p53-deficient mouse tumours, as well as 30 human retinoblastoma samples, identified members of the miR17−92 cluster as key pro-oncogenic miRNAs in retinoblastoma. High-resolution Array-CGH revealed that focal amplification of miR17−92 or a gain of the respective genomic region occurs in a subset of human retinoblastoma. Functional inactivation of the miRNAs encoded by miR17−92 cluster resulted in the death of human retinoblastoma cell lines.
Conclusions: We show that Dicer1 is required for retinoblastoma and identify Dicer1 as the first synthetic lethal partner of tumour suppressor p53. Moreover we identify members of the miR-17−92 cluster as key therapeutics targets for the treatment of retinoblastoma.

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385 Characterization and relevance of TM9SF4, a new protein associated to metastatic phenotype of human melanoma

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Background: Cannibal cells have been detected in human tumours of different histology, and their presence was always related to a poor prognosis. We have reported that metastatic melanoma cells are able to phagocytose apoptotic cells al well as live lymphocytes, with phagocytic capabilities resembling amoebas feeding process. Conversely primary melanoma cells did not show any phagocytic activity.
The amoeba *Dictyostelium discoideum* has been widely used as model to study phagocytosis and PHG1A is a key protein involved in *D. discoideum* phagocytosis. The PHG1A human closest homologue is TM9SF4 a protein completely uncharacterized until now. The aim of this study is to characterize TM9SF4 and evaluate the role of this new protein in metastatic melanomas as possible marker of malignancy.
Methods: TM9SF4 expression was evaluated by RT-PCR and Western Blot analysis (WB) of several melanoma cell lines obtained from metastatic lesions, and previously analyzed for their cannibal behavior. WB and immunofluorescence (IF) analysis were utilized to verify TM9SF4 subcellular localization. TM9SF4 functions were studied utilizing siRNAs. Acidic vesicles acidity and cytoplasmic ph were respectively evaluated by BCECF and FITC-Dextran FACS analysis. TM9SF4 expression on exosomes were evaluated by FACS and ELISA analysis.
Results: We have observed by RT-PCR, Western Blot and immunohistochemical analysis that TM9SF4 is highly expressed in malignant melanoma cells while undetectable in healthy skin and peripheral blood mononuclear cells. TM9SF4 subcellular localization experiments suggest that this protein is mainly recovered in cellular organelles, where colocalizes with early endosomal markers Rab5 and EEA1. Moreover TM9SF4 exerts a key role in the phagocytic/cannibal behaviour of metastatic melanoma cells and it is involved in the regulation of intracellular pH since TM9SF4 silencing increases cytosolic PH and induced an alkalinization of acidic endosomes. Finally TM9SF4 has been detected on exsosomes deriving from tumour cells and from exosomes out of plasma of melanoma patients, while undetectable in those deriving from healthy donors.
Conclusions: This study propose TM9SF4 as a tumour associated protein representing a new marker of malignancy that can be utilized as diagnostic and or prognostic factor, and a potential new target for anti-tumour strategies.