including GM-CSF, validated by both cytokine array and quantitative real-time PCR.
In addition, almost all endstage Siah2 knockout tumours lacked stromal infiltration. Transplant experiments demonstrated that this was caused by the inability of Siah2 knockout stroma to respond to signals secreted by advanced-stage tumour cells. These *in vivo* results, in agreement with *in vitro* results, show that stromal cells from Siah2 knockout mice cannot or minimally react to tumour cell-derived signals. Thus, these observations suggest that Siah2 could potentially regulate tumour onset and progression in a multi-faceted manner (stromal infiltration and tumour vascularisation). Siah2 displays tumour cell autonomous and stroma cell autonomous functions, suggesting the possibility of developing Siah2 as a target for anti-angiogenic therapy in breast cancer.

432 MicroRNAs in the miR-200 family differentially regulate cell cycle progression and EGF-driven invasion by modulating p27/Kip1, CDK6 and PLC-gamma1 in breast cancer

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MicroRNAs (miRNAs) in the miR-200 family are located in the fragile chromosomal regions and downregulated with tumour progression. Although members of the miR-200 family have been reported to regulate epithelial-to-mesenchymal transition (EMT) and TGF-β-driven cell invasion, there are no studies until now showing the role of individual members of the miR-200 family, especially of the miR-200bc/429 cluster, on breast cancer cell cycle progression, proliferation and EGF-driven invasion. Here, we demonstrate that miR-200 family members differentially regulate viability, cell cycle progression and EGF-driven invasion of breast cancer cells. While the miR-200a/141 cluster results in G1 arrest by increasing p27/Kip1 and downregulating CDK6 levels, the 200bc/429 cluster decreases G1 population by reducing p27/kip1 level and increasing G2/M phase potentially by reducing CDK2 and increasing Cdc2 levels. Furthermore, we have demonstrated for the first time that all miR-200 family members regulate also EGF-driven invasion, but miR-200bc/429 cluster had stronger effect compared to the miR-200a/141 cluster. Genome-wide microarray profiling in combination with gain-of-function studies identified PLCG1, which was downregulated only by the miR-200bc/429 cluster, as a potential candidate contributing to this difference. Downregulation of PLC-gamma1, whose enzymatic activity is required for EGF-induced cell motility, introduces a new role of miR-200bc/429 regulation of cell invasion besides the known TGF-β-dependent pathway. Overall, our results suggest that the miR-200 family has a tumour-suppressor function by inhibiting cell cycle progression and EGF-driven cell invasion in breast cancer.

433 Up-regulation of thymosin beta4, integrin alpha6, and cathepsin L is critical for the high invasiveness of fibrosarcoma cells

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Background: To combat against cancer-related deaths, understanding of the mechanisms behind cancer cell invasion and metastasis is of utmost importance. Mouse fibroblasts transformed by S-adenosylmethionine decarboxylase overexpression (Amdc cells) are highly invasive *in vivo* and *in vitro*, thereby providing a valuable model to study the mechanisms of cell invasion.
Materials and Methods: Gene expression changes in Amdc cells, as compared to normal NIH3T3 cells, were analyzed by DNA microarrays. Most interesting changes were confirmed by RT-PCR and Western blotting or immunofluorescence staining, and the functions of the identified molecules were then studied *in vitro* in three-dimensional cell cultures using function-blocking antibodies and specific inhibitors. Finally, the expression patterns of the identified molecules were studied in human sarcoma specimens by immunohistochemistry.
Results: We found the actin sequestering molecule thymosin β4 (Tβ4), the adhesion regulator integrin α6 (ITGα6), and the protease cathepsin L (CTSL) to be markedly overexpressed in Amdc cells. By using a sponge toxin latrunculin A (inhibiting Tβ4), function-blocking ITGα6 antibody, or CTSL inhibitor, we could block the invasion of Amdc cells in three-dimensional Matrigel. Further, we found human high-grade sarcomas to show strong ITGα6 immunostaining, especially in the invasion fronts. Tβ4 and CTSL also showed elevated immunostaining in these tissue specimens.
Conclusions: The up-regulated molecules Tβ4, ITGα6, and CTSL are important in three steps of Amdc cell invasion: migration, adhesion, and proteolysis, respectively. Inhibition of either of them suffices to block the invasion of Amdc cells, but targeting them all at the same time could give the cancer cells less chance for adaptation. Combination of Tβ4, ITGα6, and CTSL antagonists may thus show promise for the treatment of highly invasive fibrosarcomas overexpressing these molecules.

434 A role for Gsdmb in invasion and motility of Her2+ breast carcinoma cell lines

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Background: One of the molecular markers related to the aggressiveness of breast tumours is increased expression of oncogene Her2neu (ErbB2) (caused in most cases by genetic amplification). The over-expression of this oncogene occurs in around 15−30% of the most aggressive and worst prognosis primary breast cancer tumours. The co-amplification and/or co-expression of certain genes located in the same chromosomic region as Her2neu (17q12-q21) have been studied, and suggest an effect on response to treatment, or even on recurrence, of this type of tumour [1]. *Gsdmb* is a novel gene located on human chromosome 17q21 near to the Her2 amplicon. To date, Gsdmb expression has been described in gastric tumour epithelium [2]; however the functional significance of Gsdmb in cancer biology is still unknown.
Material and Methods: To analyse the hypothetical role of Gsdmb we used different approaches using two different breast tumour series and also in Her2+ breast carcinoma cell lines.
Results: Our work describe that Gsdmb amplification/over-expression occurs in a subgroup of Her+ breast carcinoma. Additionally, our data show Gsdmb cytosolic localization in breast tumour samples correlated to Her2 amplifification and local tumour recurrence. We have identified two different isoforms (named Gsdmb1 and Gsdmb2) that differ only in nine aminoacids and are mostly detected in Her2+ breast carcinoma cell lines. From the molecular point of view, we found that Gsdmb1 promotes increased phosphorylation status of ERK1/2 while Gsdmb2 increases Her2 receptor phosphorylation in specific residues, suggesting a differential role for these isoforms. Moreover, Gsdmb1 and Gsdmb2 over-expression enhances the migration and invasion of SKBR3 breast carcinoma cell line. This phenotype seems to be correlated to Rac1 activation and MMP1/14 mRNA expression.
Conclusions: Our data strongly suggest that Gsdmb1 and Gsdmb2 over-expression in Her2+ breast carcinomas increase migration and invasion of tumour cells. These results together with our data human breast tumours demonstrate that Gsdm1 and Gsdmb2 could be considered as important targets for cancer therapy.
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435 LOXL2 as a new marker of basal-like phenotype in breast cancer

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Background: Lysyl oxidase-like 2 (Loxl2) interacts with and stabilizes Snai1 transcription factor promoting epithelial-mesenchymal transition (EMT) [1]. Our recent studies showed that human LOXL2 is as a new poor prognosis indicator in human squamous cell carcinomas promoting malignant transformation by both Snai1-dependent and independent mechanisms [2]. In addition, expression profiling meta-analysis showed that high levels of LOXL2 mRNA correlated with poor prognosis in lung squamous cell carcinoma and lymph node negative (N0) breast adenocarcinomas [2], thus suggesting that LOXL2 could be involved in tumour progression.
Material and Methods: Using a high-throughput platform the expression profiling of breast carcinomas tumours (n = 59) was analyzed. Additionally, stable silencing of LOXL2 in MDA-MB-231 basal breast cancer cells was performed using sh-RNA. Cells were characterized at the morphological and behavioral levels.
Results: LOLX2 expression was correlated with basal-like breast tumours subtype, at both mRNA and protein level. Basal-like breast carcinomas are a subtype of breast tumours characterized by the negative expression of ER, PR, and Her2neu and the re-expression of different basal markers (CK5, Vimentin, FN, etc). Silencing of LOXL2 in MDA-MB-231 cancer cells leads to re-expression of epithelial markers such as E-cadherin and promotes reduced cell invasion and motility. In addition, the growth of primary tumours induced by LOXL2-silenced cells in nude mice was also reduced.
Conclusions: These results suggest that LOXL2 is involved in basal-like breast tumours progression and/or dissemination.
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