## Cancer cell plasticity in the absence of the EMT activator Zeb1

**Anna Schwinn[1], Angela Krebs[1], Dieter Saur[2], Roland Rad[3], Marc Stemmler[1]**
[1]***AG Brabletz, Experimentelle Medizin I, Nfz Erlangen, FAU Erlangen Nürnberg, Erlangen, Germany***
[2]***Technische Universität München, Klinikum Rechts der Isar II, München, Germany***
[3]***2. Medizinische Klinik am Klinikum Rechts der Isar, München, Germany***

Pancreatic cancer is currently the fourth leading cause of cancer death in Europe. Due to late detection when the tumor shows already an advanced stage, early metastasis and therapy resistance the 5-year survival rate is less than 5%. Dissemination of tumor cells is enabled by an aberrant activation of the epithelial-mesenchymal transition (EMT) program which promotes cell migration and invasion. A key inducer of EMT is the transcription factor Zeb1, which was shown to be a major regulator of cell plasticity. Acquisition of cell plasticity is mandatory for tumor cells to adapt to the hostile environment during metastasis formation. To study the role of Zeb1 in metastasis progression we used a well-established genetically modified mouse model for pancreatic cancer (KPC mice) utilizing pancreas-specific expression of mutant $Kras^{G12D}$ and $Trp53^{R172H}$ combined with simultaneous ablation of Zeb1. Isolated tumor cell lines from KPC mice ($Zeb1^{+/+}$) show a high phenotypic variability and plasticity, reflected for example by transition between epithelial and mesenchymal states. In contrast, cells derived from Zeb1-deficient tumors (KPCZ) exclusively show epithelial phenotypes which have lost cell plasticity at multiple levels. Whereas epithelial KPC cells undergo EMT by TGFβ treatment, KPCZ cells remain fixed in their epithelial state. To investigate whether presence of Zeb1 is required already during onset of tumor progression and whether Zeb1-mediated cell plasticity is reversible in established tumor cell lines we generated CRISPR/Cas9-mediated Zeb1 knockouts of various KPC cell lines. Preliminary data indicate that homozygous Zeb1 depletion in mesenchymal tumor cells partially induces epithelial characteristics. Very strikingly, Zeb1 ablation in epithelial-type KPC cells shows a strongly reduced response to TGFβ treatment compared to non-targeted controls. These data indicate that Zeb1 is not only required to provide cell plasticity at the onset of cancer, but also in established carcinoma cells. However, some residual plasticity in the CRISPR/Cas9 Zeb1 knockout clones indicates that established tumor cells are less dependent on the presence of Zeb1.