Tumor supressor gene SASH1: a new essential factor for EMT and metastasis formation?

Johannes Müller[1], Franke Fabian[1], Klaus-Peter Janssen[1]
[1]***Technische Universität München, Munich, Germany***

The protein encoded by SAM and SH3 containing 1 (*SASH1*) has recently been described as tumour suppressor in colon cancer and other solid tumours, whose expression is positively correlated with patient survival. Downregulated SASH1 was highly significantly associated with metachronous metastasis in colorectal cancer, constituting an independent prognostic parameter for survival. SASH1 is a scaffold protein from the SLy adapter family without enzymatic activity, involved in the formation of protein signalling complexes. Our recent research focuses on the involvement of *SASH1* in epithelial to mesenchymal transition (EMT), one of the major prerequisites enabling cancer cells of epithelial origin to become invasive and spawn metastases. Based on our clinical data, we hypothesize that downregulated expression of *SASH1* is necessary for EMT and metastasis formation. A straightforward approach to investigate the functions of *SASH1* is the identification of its cellular interaction partners where we concentrated on known structural SASH1 domains: The SH3 domain, which typically almost exclusively binds to PXXP motives, and the SAM domains, whose role might be protein binding as well as RNA binding. By affinity chromatography experiments based on a recombinantly expressed SH3 domain, we were able to identify new putative binding partners which are currently being analysed.

Further, we found that human HCT116 colon cancer cells downregulate mRNA and protein expression of *SASH1* during EMT, induced by TNFα. Moreover, stable depletion of *SASH1* expression via shRNA, or CRISPR-induced SASH1 deficiency induces a mesenchymal phenotype in the formerly epithelial HCT116 cells, and leads to typical hallmarks of EMT. These data raised the question whether SASH1 acts as an upstream regulator or as downstream effector protein during EMT. SASH1 might function as a central regulatory factor that coordinates protein complexes whose function is to suppress the cellular EMT signalling program. In accordance with our clinical data, reduced expression and loss of heterozygosity of *SASH1* may result in loss of this suppressor function and subsequently in EMT. Therefore, we hypothesize that intestinal epithelial cells can only undergo EMT, if *SASH1* is down regulated at the transcriptional level through an EMT activating signal. To investigate this, we established luciferase reporter systems for various regions of the regulatory region of the human *SASH1* gene, identifying a robust promoter activity immediately upstream of the transcript start site. With this model, we currently aim to identify specific loci of transcription factors, and thereby to elucidate the mechanism regulating *SASH1* in the context of EMT.

Summarised, our research aims to identify the cellular function and regulation of *SASH1*, which is known to be affected by EMT and, according to our data, is directly involved in EMT signalling and regulation.