The tumor suppressor SASH1 interacts with the signal adaptor CRKL to inhibit epithelial-mesenchymal transition and metastasis formation

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The tumor suppressor SASH1 (SAM- and SH3-domain containing 1) has clinical relevance in colorectal cancer and other solid tumor entities. It likely acts as a scaffold protein, modulating several tumor promoting signaling pathways. Notably, we could show previously that reduced expression of *SASH1* in colon cancer is significantly correlated with decreased distant metastasis free survival, indicating that SASH1 is clinically implicated in metastasis formation.

To investigate the specific impact of SASH1 on pro-metastatic functions, SASH1-deficient HCT116 colon cancer cell lines were generated using the CRISPR/Cas9-system. Loss of SASH1 induced a conversion from an epithelial morphology to a fibroblast-like appearance. Since such a conversion is typically observed for epithelial-mesenchymal transition (EMT), we further investigated the role of SASH1 in this process. Both at the mRNA and protein level, SASH1-deficient cell lines exhibited strongly reduced epithelial markers, while mesenchymal markers and EMT-promoting transcription factors were upregulated. This indicates that HCT116 cells undergo an EMT upon loss of SASH1. Moreover, using transwell assays and 3D-cell cultures, we found a strong increase in the migratory and invasive behavior of SASH1-deficient cells, as well as elevated anchorage-independent cell survival. To investigate the underlying molecular mechanism, we sought to discover potential interaction partners of SASH1 that are related to EMT. Using a combined approach of yeast two-hybrid screens and co-immunoprecipitation mass-spectrometry experiments we identified the signal adaptor and proto-oncogene CRKL as a new interaction partner of SASH1. This interaction, which was further verified by co-immunoprecipitation of endogenous proteins, could be mapped to the SH3 domain of CRKL binding to a specific motif in the C-terminal region of SASH1. In accordance, forced expression of SASH1 diminished interactions between CRKL and its effector proteins. Strikingly, loss of CRKL completely rescued the fibroblast-like morphology, mesenchymal marker expression, survival in soft agar, as well as the migrative and invasive characteristics of SASH1-deficient cells.

Based on these data, we propose that SASH1 acts as an inhibitor of CRKL-mediated signaling, which promotes EMT and likely metastasis formation *in vivo*, through a direct physical interaction. We are currently investigating the role of the novel interaction between SASH1 and CRKL in an *in vivo* mouse model.