The role of the adaptor protein CKRL in EMT and metastasis

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The oncoprotein CRKL (v-CRK avian sarcoma virus C10 regulator of kinase–like) is a signal adapter protein containing one SH2 and two SH3 domains. It has previously been described as an oncogene, and participates in the activation of the Ras-signaling pathway. In this project, we are investigating a direct protein-protein interaction between SASH1, a tumor suppressor with clinical importance in colorectal and lung adenocarcinoma, and the oncoprotein CRKL. As working hypothesis, we propose that SASH1 inhibits CRKL function, and this interaction is important for the control of the phenotypical change from the epithelial to the mesenchymal state, the so-called epithelial-mesenchymal transition (EMT).

To investigate this issue, we have successfully created CRKL deficient cell lines, based on HCT116 colon cancer cells, and are currently implementing CRKL-deficiency in A549 lung adenocarcinoma cells, using the CRISPR/Cas9 system. The respective clones are currently analyzed for their phenotype in vitro and in vivo. After confirming the CRKL-deficiency by direct DNA sequencing, as well as on mRNA and protein level, the effects of serum starvation and re-stimulation on the phosphorylation of important growth signal cascades are investigated, e.g., for the Ras-MAPK pathway.

Besides that, experiments were performed to shed light on the ability of HCT116 cells to undergo EMT after treatment with tumor necrosis factor alpha (TNFα). Phenotypic changes were observed via immunofluorescence microscopy and immunoblot analysis. CRKL-deficient HCT116 cells failed to undergo EMT after treatment with TNFα, compared to parental cells. This was evidenced by analysis of the level of the cell-adhesion protein E-Cadherin, and the expression of EMT-inducing transcription factors of the SNAIL family (SNAIL, SLUG) and ZEB1. Further, increase of MAPK activity after serum restimulation was partially impaired after CRKL deficiency.

Currently, we are analyzing A549 derived cells and their ability to undergo EMT after treatment with tumor growth factor beta (TGFβ), as well as their reaction to serum starvation and re-stimulation.

In addition to altered MAPK-signaling, the regulation of cell-matrix adhesion is a major focus of this project. The ability of HCT116 parental or CRKL deficient cells to successfully attach to a fibronectin coated plate was investigated in kinetic experiments. The matrix-adhesion is important for cancer cells that migrate through tissue, and plays a pivotal role in local invasion of nearby tissue, as well as for distant metastasis. Our investigations show that CRKL deficient cells show decreased adhesion to fibronectin, and have a deficiency in integrin dependent signaling. Taken together, our findings suggest that CRKL is an essential protein for EMT of cancer cells.