Abstract

CRN2 is a member of the coronin family of proteins which belongs to the super-family of WD40-repeat domain proteins. It is localized to cell areas characterized by dynamic actin remodeling, where it plays a role in actin bundling and inhibition of actin polymerization. In both cases CRN2 is negatively regulated by CK2 which phosphorylates serine 463 of CRN2. Additionally, CRN2 expression is associated with the malignant phenotype of diffuse gliomas as well as initiation of pulmonary metastasis in hepatocellular carcinoma. In order to address the relevance of CRN2 for tumor progression, stably transduced human glioblastoma cell lines with a shRNA mediated knock-down of CRN2 or an over-expression of GFP-tagged CRN2 fusion proteins were generated. These cell lines were used in various in vitro assays to analyze cell proliferation, migration, invasion, and protrusion formation. Moreover, they were transplanted on organotypic brain slice cultures, which served as a more physiological ex vivo model of tumor cell invasion. Analyses of the size and invasion rate of transplanted tumors showed increased invasion and a highly diffuse morphology for cells over-expressing CRN2, and decreased invasion and round morphology for the CRN2 knock-down cells. Furthermore, a reduced tumor cell invasion in cells expressing the S463D phospho-mimetic CRN2 variant as compared to cells expressing the S463A phospho-resistant CRN2 variant was observed. Importantly, differences in the F-actin distribution in invadopodia-like cell extensions were determined. Glioblastoma cells expressing the phospho-resistant CRN2 variant exhibited a centrally enriched pattern of actin, while cells expressing the phospho-mimetic variant or lacking CRN2 showed an accumulation of actin at the rim zone of the cell extension or diffuse distribution of actin, respectively. This may led to a less stable F-actin network in invadopodia-like extensions, which subsequently would decrease the invasiveness of these cells. In order to analyze the biological role of CRN2, knock-out mouse lines with reporter insertion, conditional, reporter deletion, and deletion alleles were generated. The knock-out was validated via PCR, Southern blot and western blot. First analysis of the CRN2 knock-out mice revealed an increased anxiety phenotype. Additionally, defects in the formation of F-actin stress fibers and the formation of cellular protrusions as well as a reduced migration velocity were found in CRN2 knock-out primary skin fibroblasts.