

Abstract

CRN2 is an actin filament binding protein that has been shown to be involved in processes like protrusion formation, cell migration and invasion. It has been implicated in the progression of different types of cancer like melanoma, gastric cancer, hepatocellular carcinoma, primary effusion lymphoma and glioblastoma. In order to analyse the functional role of CRN2, we generated and comprehensively characterised CRN2 knock-out mice, which, however, did not develop any conclusive phenotype. To investigate putative effects of the CRN2 ablation at the cellular level, we isolated and investigated primary skin fibroblasts. Beside other changes, they displayed an aberrant organisation of the vimentin intermediate filament network in conjunction with mitochondrial alterations that could be restored upon ectopic CRN2 overexpression. Furthermore, we identified vimentin as a novel binding partner of CRN2. Both the β -propeller and the coiled coil domain of CRN2 interacted with the non- α -helical “head” domain of vimentin. Based on our and other observations that coronin proteins can localise and bind to actin filaments, intermediate filaments and microtubules, we hypothesise that CRN2 functions as a novel versatile linker of the cytoskeleton.

Supporting previous reports, we provide several lines of evidence that the expression of CRN2 increases the malignant phenotype of human gliomas. Here, we could show a higher encasement rate of murine brain slice tissue capillaries by transplanted glioblastoma tumour cells overexpressing CRN2 as well as focal adhesions of a smaller size in these cells as compared to CRN2 knock-down glioblastoma cells. Furthermore, we used glioblastoma mouse models without and with the additional knock-out of CRN2 to characterise the biological role of CRN2 in glioblastoma progression. Though there are no obvious differences in the life span of these mice after glioblastoma induction, we found smaller tumours in the initial growth phase of the glioblastoma in mice with the additional knock-out of CRN2.

Notably, we identified the tissue inhibitor of matrix metalloproteinases 4 (TIMP4) and the matrix metalloproteinase 14 (MMP14), which are important factors of the tumour microenvironment, as novel CRN2 binding partners. All three proteins mutually interacted and showed a partial co-localisation at the front of lamellipodia. Domain mapping of the interaction sites revealed binding of CRN2 to the N-terminal domain of TIMP4 and to the catalytic domain of MMP14. We further could demonstrate that the latter interaction enhances the catalytic activity of MMP14.

Taken together, our results expand the spectrum of CRN2 interaction partners, link CRN2 to the tumour microenvironment and clearly highlight a contribution of CRN2 to the malignant progression of glioblastoma.