

SUMMARY

The adaptor protein p130Cas (CAS, BCAR1) represents a nodal signaling platform for integrin and growth factor receptor signaling, and influences normal development and tissue homeostasis. Its altered expression drives many pathological conditions including tumor growth, metastasis and drug resistance in many cancer types. How p130Cas contributes to many of these pathologies is still poorly understood. Therefore, the overall aim of my PhD work was to provide new insights to p130Cas signaling and its regulation.

The SH3 domain is indispensable for p130Cas signaling, but the ligand binding characteristics of the p130Cas SH3 domain, and the structural determinants of its regulation were not well understood. To be able to study various aspects of p130Cas signaling we identified an atypical binding motif in p130Cas SH3 domain by establishing collaborations with Dr Veverka (Structural biology) and Dr Lepšík (Computational biochemistry; Academy of Sciences, CZ) which gave new insight into this binding interface. Through these collaborations I generated chimeras of p130Cas SH3 domain with its ligands for structural NMR analysis and learned how to visualize and analyze structures. Furthermore, my work expanded our knowledge of p130Cas SH3 ligand binding regulation and led to a novel model of Src-p130Cas-FAK binding. Our efforts to monitor Src kinase activity and conformation, which influence how Src phosphorylates p130Cas, led us to construct a novel FRET-based Src biosensor and allowed me to use cutting edge FRET imaging.

In addition, I developed a bioinformatic workflow to identify novel direct p130Cas binding partners that led to identification DOK7, GLIS2 and PKN3, and helped to validate direct binding to Vinculin. Vinculin-p130Cas interaction was further demonstrated to have a crucial role in mechanosensing and focal adhesion dynamics, and the interaction with DOK7 and GLIS2 gave insight into neuromuscular and kidney pathologies. Finally, I helped to demonstrate that p130Cas interacts with Ser/Thr kinase PKN3 in pro-invasive cell invadopodia/lamellipodia and showed that this interaction is important for mouse embryonic fibroblast growth and invasiveness independent of Src transformation, indicating a mechanism distinct from that previously suggested for p130Cas. I further showed the relevance of this work to human breast cancer combining *in vitro* work with analysis of proteomic data to provide evidence that PKN3 phosphorylates p130Cas in invasive breast tumors.