ABSTRACT

Focal adhesions are important subcellular structures that are composed of many signaling and scaffolding proteins. They serve not only for anchoring the cell to the substratum but they are also important signaling centers that regulate various cellular behavior such as migration, invasiveness, proliferation and survival. Focal adhesion signaling needs to be strictly regulated because alteration in activity or expression of many focal adhesion proteins leads to tumorogenesis and metastasis formation. One of the most important scaffolding protein associated with focal adhesion is p130Cas. The importance of p130Cas in regulation of cell migration and invasiveness has been well established. P130Cas also plays important role in regulation of cell survival and proliferation. Moreover, high protein levels of human ortholog of p130Cas – BCAR1, has been linked to more aggressive breast tumors and poor prognosis.

During my doctoral studies, I focused on the role of p130Cas in integrin signaling. At the beginning we characterized the role of tyrosine 12 phosphorylation within its SH3 domain. We confirmed that this phosphorylation is increased in Src527F transformed mouse embryonic fibroblasts compared to non-transformed counterparts and also in some human cancer cell lines. We showed that this phosphorylation disrupts binding capacity of p130Cas SH3 domain and alters phosphorylation levels of some focal adhesion proteins. Furthermore, phosphomimicking mutation Y12E leads to delocalization of p130Cas from focal adhesion, but expression of this variant increases the migration and invasiveness of mouse embryonic fibroblast. This effect was probably caused by increased dynamics of focal adhesion in cells expressing p130Cas Y12E variant. Moreover, expression of non-phosphorylatable mutation of p130Cas (Y12F) has oposite effect on focal adhesion dynamics, migration and invasiveness.

Subsequently, we identified vinculin as a novel binding partner for p130Cas SH3 domain and confirmed that this interaction is direct. We showed that this interaction is important for proper localization of p130Cas to focal adhesion and influences the size and dynamics of adhesion structures. Moreover, we found that p130Cas-vinculin interaction is important for stretch induced phosphorylation of p130Cas substrate domain and the phosphorylation of Y12 within p130Cas SH3 domain abolished mechanical activation of p130Cas. This interaction has been found to be important also for maintaining proper cell stiffness and generation of traction forces.

Finally, during my study I also participated in characterization of assembly and dynamics of focal adhesion formed by cells grown in 3D environment.