Characterization of cortactin nanobodies specific for its NTA domain

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Abstract

Cortactin has been shown in numerous studies to promote cell migration and invasion. A key event in the process of invasion is the formation of actin filaments that build up invadosomes. Invadosomes are structures capable of degrading the extracellular matrix through secretion of matrix metalloproteases (MMPs). Cortactin (cortical actin binding protein) is a c-Src substrate and a marker for invadosomes. Even though it is clear that cortactin is essential for invadosome formation and maturation, the exact contribution of the different cortactin domains remains unclear. Some studies claim that the C-terminal SH3 domain is crucial whereas others suggest that cortactin phosphorylation or NTA (N-terminal acidic) domain mediated activation of the Arp2/3 complex is of key importance. We use Cameloid single domain antibodies or nanobodies that bind the NTA domain to clarify the role of the N-terminal cortactin region in invadosomes. These data are compared to previously obtained results with a nanobody that binds the cortactin SH3 domain.

Nanobodies were generated through immunization of an alpaca with a cortactin fragment containing the NTA domain and actin binding repeats. Several rounds of phage panning and ELISA yielded two nanobodies. These two NTA nanobodies bind cortactin fragment A that harbors the NTA domain but not with fragment B that lacks the NTA domain. They are able to pull down cortactin from MDA-MB-231 cell lysates and disturb the interaction between the NTA domain and the Arp2/3 complex. This interaction remains unaffected in the presence of the SH3 nanobody. After stable expression in cell lines, the NTA nanobodies colocalize with cortactin and invadosomes. Quantification of invadosome formation demonstrates that NTA nanobody expression increases invadosome number and spread area, while a nanobody that binds the SH3 domain has the opposite effect. Future research will focus on the effect on MMP secretion and cell migration and invasion.