A FASCINating view on podosome dynamics by means of nanobodies

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Abstract

Podosomes are dynamic degrading devices present in cells of myeloid lineage. They consist of an actin-rich core with several actin regulators surrounded by an adhesive ring. Their counterparts in cancer cells, called invadopodia, contain the same proteins but differ in actin arrangement and structure. Fascin, an actin bundling protein, is considered a metastatic marker and an invadopodium component contributing to actin stabilization, matrix degradation and invasion. Although fascin is also known to be a component of podosomes, its precise function in these structures remains unclear.

A fascin nanobody (FASNb5) able to inhibit fascin actin bundling was used as a unique tool to study fascin contributions in podosome formation. Nanobodies represent the antigen-binding fragments of *Camelid* heavy-chain antibodies and are an emerging tool in research due to their small size, stability, solubility, affinity and specificity.

Upon FASNb5 expression in THP-1 macrophages, only limited amounts of podosomes are formed in comparison to control conditions. Moreover, the podosomes are aberrantly stable, long-living and big, suggesting a role for fascin actin bundling in podosome turnover and disassembly. The podosome-loss is compensated by the presence of focal adhesion structures and therefore only modestly affects matrix degradation capacities. Development of a migratory phenotype however strongly depends on podosomes and their flexibility. FASNb5 expression therefore results in impaired generation of an elongated polarized phenotype in both THP-1 cells and primary DC.

Further experiments show that fascin regulates its podosome disassembling role at a cap-like localization by modulating its expression levels relative to actin. By increasing fascin levels, actin building blocks switch from Arp2/3-dependent branches towards more unbranched fascin bundles causing podosome turnover.

In conclusion, we were able to show by means of nanobodies that fascin actin bundling is a master regulator of podosome dynamics.