Abstract

Netrins belong to a family of phylogenetically conserved guidance molecules. Their functions have been extensively studied in the development of the nervous system, where they control multiple neuronal growth and migration activities. Depending on the receptors expressed on their growth cones, axons can either be attracted or repelled by netrins. The expression of netrins is, however, not restricted to the nervous system. Netrins are also involved in the morphogenesis of several organs such as lung, intestine, pancreas, and mammary gland.

In mammals five netrins have been identified: netrin-1, netrin-3, netrin-4, netrin-G1, and netrin-G2. For two of those, netrin-1 and nertin-3, binding to receptors from the DCC- and UNC-5-family has been described. In this study the interactions of two additional netrins, netrin-4 and netrin-G1, with the receptors DCC, neogenin, UNC5B and UNC5C were analysed by Biacore binding studies. In contrast to netrin-1, netrin-4 binds neither to DCC nor to neogenin. However, both netrin-4 and netrin-G1 bind to the receptor UNC5C. Unlike the interaction with UNC5C, the interaction of netrin-4 and netrin-G1 with the receptor UNC5B could only be detected when the receptor was expressed as dimer by adding the Fc fragment of human IgG.

In addition to the binding analysis of netrin-4 to known netrin receptors, binding to unknown ligands was explored. Netrins are structurally related to the N-terminal domains VI and V of laminin chains. Laminins, a major component of basement membranes, build a network through their N-terminal VI domains. Since netrin-4 was primarily found in the basement membranes of several organs, possible interactions of netrin-4 with laminin chains were analyzed. A particularly strong binding between netrin-4 and the laminin γ1 and γ3 chains was identified. Remarkably, this interaction could only be detected with netrin-4, but not for netrin-1 or for netrin-G1.

To study the functional role of netrin-4 on basement membrane formation, the effect of netrin-4 on salivary gland branching morphogenesis and laminin polymerization was analyzed. These *in vitro* experiments demonstrated that netrin-4 is a potent inhibitor of salivary gland branching and also laminin polymerization is clearly influenced by the addition of netrin-4.

For the detailed understanding of netrin-1 and netrin-4 interactions with their respective receptors and extracellular matrix ligands it is crucial to elucidate their 3D structure. Therefore with both proteins crystallization experiments were performed and the crystal structure of netrin-1 is near completion.