Abstract

Angiogenesis is one of the major physiological processes in the human organism as the functional vascular system is essential for providing an efficient delivery of oxygen and nutrients to discrete tissues. In this context a stringent regulation of the blood vessel homoeostasis via pro- and anti-angiogenic factors is crucial.

The extracellular matrix protein netrin-4 is supposed to be one of these regulatory factors in angiogenesis, however, it is controversially discussed, whether its effect is pro- or anti-angiogenic and which cellular receptor is responsible for mediating its action. In addition, the active domain within netrin-4 is unknown. Therefore the main goal of this study was to analyse the function of netrin-4 in the angiogenic process of endothelial cells and furthermore to identify the active domain of netrin-4.

In *in vitro* tube formation assays of endothelial cells, the application of high doses of netrin-4 led to an inhibitory effect on tubulogenesis without triggering cell toxicity. Furthermore, netrin-4 had a negative influence on preformed tubes. Interestingly, the inhibitory effect was matrix dependent since the addition of netrin-4 resulted in reduced adhesion and spreading of endothelial cells exclusively on matrigel and laminin gel. Moreover, elongated incubation times of netrin-4 led to an inhibition of cellular proliferation and migration. To further investigate this inhibitory effect, electron microscopy studies were performed, which revealed a disruption of the endothelial basement membrane on matrigel. In addition to that, netrin-4 was not only capable to inhibit the polymerisation of matrigel and laminin gels, but also to dissolve already polymerized gels.

Testing mutants swapping particular domains of netrin-4 with corresponding structures of the related protein laminin $\gamma 1$ the active domain of netrin-4 could be identified as a combination of the LN- and the LE-domain. Via sequence analyses and molecular modeling an amino-acid sequence within the LE-domain could be determined to be unique for netrin-4. This region contributes to the formation of a huge loop comprising two antiparallel beta-sheets, which together with the globular LN-domain mediate the strong binding to endothelial laminin $\gamma 1$.

Altogether, these data identified for the first time the active domain of netrin-4 that exhibits an anti-angiogenic effect on endothelial cells. Moreover, the interaction of high concentrations of netrin-4 with laminin $\gamma 1$ leads to a destabilisation of the endothelial basement membrane and therefore negatively influences basement membrane-dependent cellular processes like adhesion, proliferation, and migration of endothelial cells.