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Midkine is important in neurogenesis and is recognized both by chondroitin sulfate proteoglycans and heparan sulfate proteoglycans

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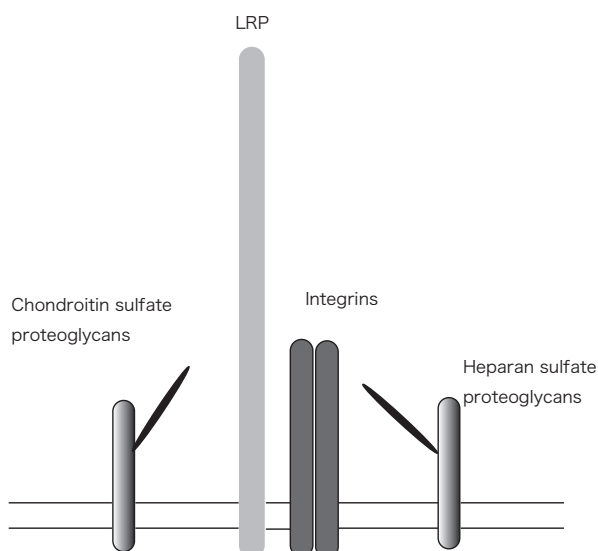


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Fig. 1. Proteoglycans in midkine signaling



Midkine is a heparin-binding growth factor or cytokine and is strongly expressed in the embryonic period; in the adult, midkine expression is induced upon tissue injury and repair and also in inflammatory and malignant diseases. One principal role of midkine in the embryonic period is to promote neurogenesis. Midkine enhances neurite outgrowth and survival of neurons. Midkine knockout mice exhibited auditory abnormality probably due to deficits of the nerve tissue in the inner ear. Midkine is also involved in the early stage of neurogenesis. In late gastrula embryos of *Xenopus*, midkine is expressed in neural anlagen. Injection of midkine RNA into vegetal blastomeres of the 8 cell stage

embryos leads to hyperproliferation of the nervous tissue and poor development of mesoderm-derived tissues. The zebra fish has two species of midkine molecules due to gene duplication. Knockdown of a midkine molecule in the fish results in poor development of the medial floor plate, which organizes the specification of neurons and outgrowth of axons in the ventral spinal cord.

The role of midkine in early stage of neurogenesis was studied using neural progenitor cells (neural stem cells and the daughter cells on the way of differentiation) from the embryonic mouse brain. Immunohistochemical staining revealed that midkine was expressed in neural progenitor cells. When neurospheres mainly consisting of neural stem cells were formed from dissociated embryonic brain cells of midkine knockout mice, their numbers were significantly smaller compared to the neurospheres from the wild-type embryonic brain. When neurospheres were dissociated by trypsin digestion and were cultured on a substratum, they yielded nestin-positive neural progenitor cells and differentiated neuronal cells. On a substratum coated with poly-L-lysine, neural progenitor cells from the brain of knockout mice spread poorly and grew and survived less effectively compared to the cells from the wild-type brain. However, on a substratum coated with midkine, neural progenitor cells from the deficient brain and those from the wild-type brain spread, grew and survived similarly. These results suggest that enhancement of growth and survival of neural progenitor cells is the basis of promotion of early neurogenesis by midkine.

The midkine receptor is a multi-molecular complex containing proteoglycans; low density lipoprotein receptor-related protein (LRP) and integrin $\alpha 4\beta 1$ or

$\alpha 6\beta 1$ play central roles (Fig. 1). For strong binding to midkine, the glycosaminoglycan portion is required to have oversulfated structures, namely chondroitin sulfate E structure or heparan sulfate enriched in heparin trisulfated units. Among proteoglycans, the best studied as the component of the receptor is receptor-type protein tyrosine phosphatase ζ (PTP- ζ). Midkine binds to its chondroitin sulfate portion with high affinity and protein portion with low affinity. PTP ζ is involved in migration and survival of embryonic neurons. Neurite outgrowth of embryonic neurons, however, requires heparan sulfate proteoglycans. cDNA transfection experiments revealed that both syndecan-3 and glypican-2 were capable of enhancing neurite outgrowth. However different cell-surface localization of the two molecules suggested their different roles in neurite outgrowth. Midkine-dependent neurite outgrowth of oligodendrocyte precursor-like cells was enhanced by neuroglycan C, a part time chondroitin sulfate proteoglycan. Presence of chondroitin sulfate enhanced the binding of neuroglycan C to midkine.

To examine which types of proteoglycans are involved in midkine-dependent growth and survival of neural progenitor cells, these cells were digested by heparitinase or chondroitinase ABC. Although neural progenitor cells expressed both syndecans and PTP ζ , only heparitinase abolished the effect of midkine. The result indicates that in neural progenitor cells heparan sulfate proteoglycans are important as the receptor component in receiving growth and survival signal of midkine. It is likely that a proteoglycan is selected for a function in a given cell as a receptor component of midkine, but the mechanism of such selection remains to be an interesting question for future research.

Keywords : Chondroitin sulfate proteoglycans, Heparan sulfate proteoglycans, Midkine, Neural stem cells, Neurogenesis