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# Syndecan-4 as a molecule involved in defense mechanisms

Kazuhiro Ishiguro[1,2], Tetsuhito Kojima[3] and Takashi Muramatsu[1]

[1]*Department of Biochemistry and* [2]*Department of Internal Medicine, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan,* [3]*Department of Medical Technology, Nagoya University School of Health Sciences, 1-1-20 Daiko-Minami, Higashi-ku, Nagoya, Aichi 461-8673, Japan*

**Syndecan-4 is a transmembrane heparan sulfate proteoglycan belonging to the syndecan family. Syndecan-4-deficient [(*Synd4*(−/−)] mice were produced to clarify the *in vivo* role of syndecan-4. *Synd4*(−/−) mice were more susceptible to κ-carrageenan-induced nephropathy, and the placental labyrinth from the deficient embryos exhibited more thrombi than wild-type ones. Importantly, *Synd4*(−/−) mice were more susceptible to endotoxin shock. Further analysis revealed that the mechanism to suppress excessive production of interleukin-1β (1L-1β) by transforming growth factor-β (TGF-β) was impaired in the deficient mice. TGF-β, one of the cytokines involved in the suppression mechanism, bound to heparan sulfate chain of syndecan-4, which was induced in macrophages and the microvasculature after administration of lipopolysaccharide. Therefore, augmentation of TGF-β function by induced syndecan-4 was suggested as a mechanism of the suppressive action of syndecan-4 against endotoxin shock.**
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## Introduction

Syndecan-4 is a member of the syndecan family, which consists of 4 transmembrane heparan sulfate proteoglycans, and is also called ryudocan or amphiglycan [1–3]. Its core protein is 30 kDa, and it has three glycosaminoglycan attachment sites in the extracellular domain (Figure 1) [1]. The extracellular domain and cytoplasmic domain have about 70% identity to those of syndecan-2, while their identity to syndecan-1 and -3 is less [4]. Syndecan-4 was found in rat microvascular endothelial cells as an antithrombin-binding molecule [1,3]. It also binds to basic fibroblast growth factor, midkine and tissue factor pathway inhibitor via its heparan sulfate chain [5]. Syndecan-4 is expressed in most adhesive cells, and the mode of its expression is different from those of other syndecans [6–8]. Syndecan-4 is localized in focal adhesions in fibroblasts and is implicated in focal adhesion formation [9–11, see accompanying article by Wilcox-Adelman et al.]. To clarify the *in vivo* role of syndecan-4, we produced syndecan-4-deficient [*Synd4*(−/−)] mice [12].

To whom correspondence should be addressed: Takashi Muramatsu, Department of Biochemistry, Nagoya University, School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. Tel.: +81-52-744-2059; Fax: +81-52-744-2065; E-mail: tmurama@med.nagoya-u.ac.jp

## General properties of *Synd4*(−/−) mice

*Synd4*(−/−) mice were produced by gene targeting to delete exon II, III and part of IV, which encode the ectodomain with three putative glycosaminoglycan attachment sites [12] (Figure 2). *Synd4*(−/−) mice were born, reproduce, and the mode of their growth is not different from that of the wild-type [*Synd4*(+/+)] control mice. No gross abnormality was found in *Synd4*(−/−) mice by histological examination [12].

At first, we examined the precise role of syndecan-4 in focal adhesion formation [12]. Focal adhesions are macromolecular complexes found at the sites of cell adhesion to extracellular matrices. Focal adhesions are linked to actin stress fibers and also serve as signaling complexes involved in triggering intracellular signaling cascades. For the formation of focal adhesions, two signals delivered by fibronectin, namely from the cell-binding site and the heparin-binding site, are recognized by two molecules on the surface of fibroblasts, integrins and heparan sulfate proteoglycans, and this dual recognition is essential for focal adhesion formation. Syndecan-4 was concluded to be the latter molecule [9–11]. We cultured fibroblasts from *Synd4*(−/−) embryos, and found that focal adhesions were formed even in *Synd4*(−/−) cells. Therefore, the loss of syndecan-4 was compensated for by other heparan sulfate proteoglycans. However, when focal adhesions were

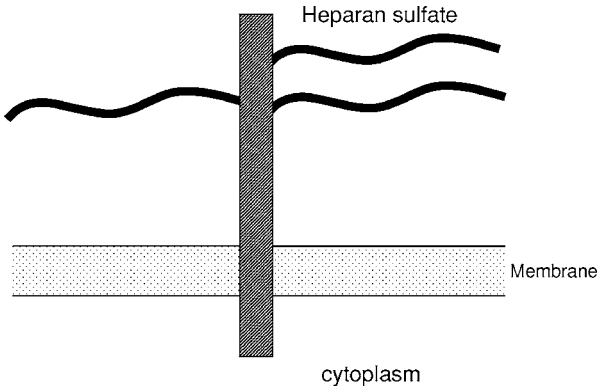


**Figure 1.** A model of syndecan-4 structure.

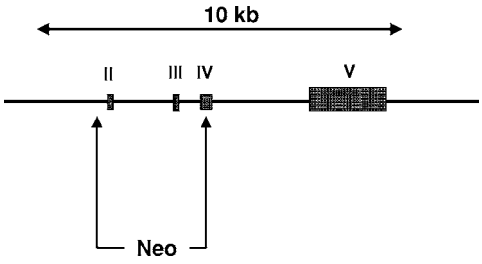


**Figure 2.** Deletion of a part of syndecan-4 gene by homologous recombination. ■, exons; II–V, exon II–exon V. The region between the arrows was deleted by replacement with *Neo* gene.

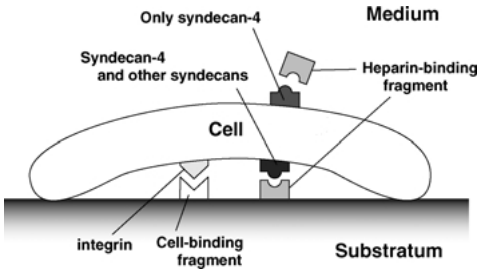


**Figure 3.** Syndecan-4 is the sole heparan sulfate proteoglycan recognized by the heparin-binding fragment added into culture medium.

artificially formed by culturing the cells on the cell-binding fragment of fibronectin and the heparin-binding fragment was added to the medium, focal adhesions occurred in *Synd4*(+/+) cells but were inhibited in *Synd4*(−/−) cells (Figure 3). In a similar experiment, in which the heparin-binding fragment was supplied in a substratum-bound form, focal adhesion formation in *Synd4*(−/−) cells was not impaired. Thus, syndecan-4 was concluded to be the sole molecule recognized by the heparin-binding site of fibronectin in the medium side (Figure 3). Upon inflammation or during wound healing, fibronectin is degraded to yield the soluble heparin-binding fragment, and syndecan-4 is expressed in the affected region. Therefore, it is possible that syndecan-4 is involved in the processes of inflammation and repair in response to a signal delivered by the heparin-binding fragment of fibronectin [12].

Syndecan-4 was originally found as a molecule implicated in anti-coagulation [1]. Since multiple molecular species are present as cell-surface heparan sulfate proteoglycans, we questioned whether deletion of a single molecular species, namely syndecan-4, leads to thrombus formation. *In situ* hybridization and immunohistochemical analysis revealed that fetal vessels in the placental labyrinth expressed syndecan-4, but not other syndecans (syndecan-1, -2 and -3) [13]. Examination of placental labyrinth formed by *Synd4*(−/−) embryos revealed that thrombi were more frequently found in the vessel formed by the embryos than those by *Synd4*(+/+) embryos. Thus, deletion of a single syndecan molecular species actually leads to increased thrombus formation in a specific tissue.

When $\kappa$-carrageenan is intraperitoneally injected, it is deposited in collecting tubules and causes obstructive nephropathy. Since syndecan-4 is strongly expressed in the collecting ducts and renal tubules in the papilla and the medulla, we examined the effect of syndecan-4 deficiency on the susceptibility to the obstructive nephropathy [14]. Indeed, we found that more *Synd4*(−/−) mice died than *Synd4*(+/+) mice by $\kappa$-carrageenan nephropathy. Larger amounts of $\kappa$-carrageenan were deposited in the collecting ducts of *Synd4*(−/−) mice than those of *Synd4*(+/+) mice. Most probably, heparan sulfate chains of syndecan-4 bind to some positively charged material and prevent deposition of $\kappa$-carrageenan, which is an acidic substance.

Echtermyer et al. also produced *Synd4*(−/−) mice and demonstrated that syndecan-4 deficiency impairs the healing of skin wounds [15, see accompanying article by Wilcox-Adelman et al.].

## Syndecan-4 in septic shock

Septic shock is sepsis with hypotension, and is a common cause of death in patients in intensive care units. Lipopolysaccharide, one of the toxins of Gram-negative bacteria, is a classic cause triggering septic shock [16].

When a relatively high dose of lipopolysaccharide (5 and 10 mg/kg) was administered, the mortality of *Synd4*(−/−) mice was found to be significantly higher than that of *Synd4*(+/+) mice [17] (Figure 4). Systolic blood pressure was abnormally low in *Synd4*(−/−) mice at 9 h after lipopolysaccharide administration. Left ventricular M-mode echocardiography revealed that left ventricular fractional shortening was significantly lower in *Synd4*(−/−) mice than *Synd4*(+/+) mice. Therefore, *Synd4*(−/−) mice are more susceptible to endotoxin shock than *Synd4*(+/+) mice. In other words, syndecan-4 is involved in a mechanism that prevents septic shock. Then, we investigated such a mechanism.

Proinflammatory cytokines, such as tumor necrosis factor (TNF)-$\alpha$ and interleukin (IL)-1$\beta$, are produced in response to bacterial infection or lipopolysaccharide administration, and play crucial roles in the cascade leading to endotoxin shock [18,19]. Thus, we determined the concentration of TNF-$\alpha$ and IL-1$\beta$ in the plasma after lipopolysaccharide administration.

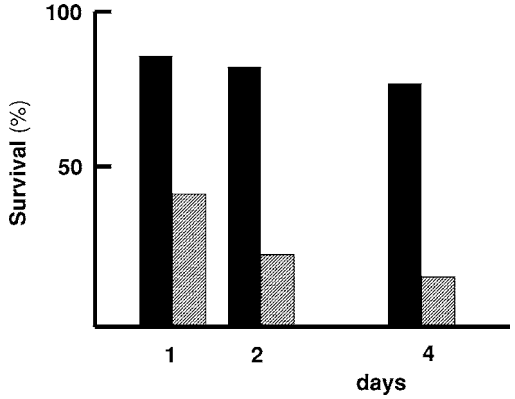


**Figure 4.** Increased mortality of *Synd4*(−/−) mice (▨) over that of *Synd4*(+/+) mice (■) after administration of 10 mg/kg lipopolysaccharide. No mice died after 4 days. Based on Ref. [17].

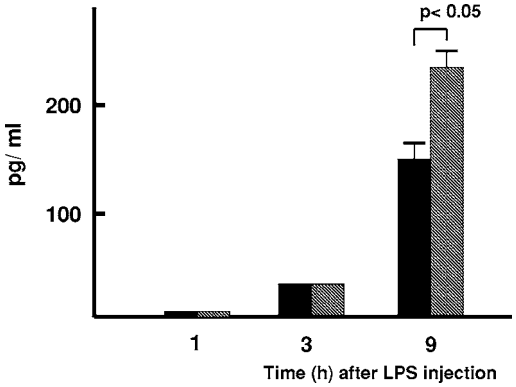


**Figure 5.** Comparison of plasma IL-1$\beta$ levels after administration of 10 mg/kg lipopolysaccharide to *Synd4*(−/−) mice (▨) and *Synd4*(+/+) mice (■). No IL-1$\beta$ was detected 1 h after lipopolysaccharide injection. Based on Ref. [17].

The TNF-$\alpha$ levels were not different between the two genotypes. However, the IL-1$\beta$ level was significantly higher in *Synd4*(−/−) mice than *Synd4*(+/+) mice at 9 h after lipopolysaccharide administration, while it was not different at 3 h (Figure 5) [17]. Therefore, a mechanism to suppress excessive increase of IL-1$\beta$ levels is impaired in *Synd4*(−/−) mice. We found that syndecan-4 expression was dramatically increased 3 h and 9 h after lipopolysaccharide administration in macrophages and microvascular endothelial cells in the lung, intestine and liver [17]. The increased expression may well be correlated with the suppressive mechanism. TGF-$\beta$ is known to participate in prevention of endotoxin shock mainly by inhibiting the production of proinflammatory cytokines [19,20]. IL-1$\beta$ production by macrophages is suppressed by TGF-$\beta$. We found that the suppression by TGF-$\beta$ was impaired in *Synd4*(−/−) macrophages. One hypothesis to explain the role of syndecan-4 is that TGF-$\beta$ binds to heparan sulfate chains of syndecan-4, leading to an increased activity of this cytokine. Indeed, we observed that TGF-$\beta$ bound to syndecan-4 via its heparan sulfate chains [17].

Our finding that syndecan-4-deficient mice are more susceptible to endotoxin shock provides one explanation for syndecan-4 being conserved among mammals. Furthermore, syndecan-4 mutation must be kept in mind in exploring the possible genetic factors influencing the susceptibility to septic shock in patients. Finally, all *in vivo* functions of syndecan-4 so far clarified are related to defense to infection or injury.

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