

Increased syndecan-4 expression in sera and skin of patients with atopic dermatitis

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Abstract Syndecan-4 (SDC-4) is a cell surface proteoglycan, which participates in signaling during cell adhesion, migration, proliferation, endocytosis, and mechanotransduction, and is expressed on various cells, including endothelial cells, epithelial cells, T cells, and eosinophils. Emerging evidences have suggested that SDC-4 might contribute to Th2-driven allergic immune responses. Here, we examined the role of SDC-4 in patients with atopic dermatitis (AD). Serum SDC-4 levels in AD patients were significantly higher than in healthy individuals, and they increased according to the disease severity. Importantly, they positively correlated with Eczema Area and Severity Index and itch visual analogue scale scores. Furthermore, serum SDC-4 levels decreased after treatment. We also analyzed SDC-4 expression in AD lesional skin. SDC-4 mRNA levels in AD skin were significantly higher than those of normal skin. Immunohistochemical staining revealed that SDC-4 was highly expressed in the epidermis and endothelial cells in AD lesional skin. Taken together, our study has demonstrated that SDC-4 expression was increased in sera and skin of AD patients, suggesting that SDC-4 may contribute to the development of AD.

Keywords Syndecan-4 · Atopic dermatitis · Allergy · Blood endothelial cells

Abbreviations

SDC	Syndecan
AD	Atopic dermatitis
EASI	Eczema area and severity index
VAS	Visual analogue scale
DC	Dendritic cell
mRNA	Messenger RNA
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase

Introduction

Syndecans (SDCs) are type-I transmembrane heparin sulfate proteoglycans comprising four family members [9]. SDC-4 participates in signaling during cell adhesion, migration, proliferation, endocytosis, and mechanotransduction [9] and is expressed on various cells, including blood endothelial cells, epithelial cells, T cells, and eosinophils [2, 11, 12]. The cytoplasmic portion of SDC-4 interacts with focal adhesion molecules, suggesting a role for SDC-4 in anticoagulation, cell adhesion, and proliferation [9]. SDC-4 also contains an extracellular domain and its shedding is accelerated under certain conditions, such as wound healing, acute inflammation, or mechanical stress [15]. Emerging evidences have suggested that SDC-4 might contribute to Th2-driven allergic immune responses. SDC-4-dependent signaling induced eosinophil migration in linkage with anti-thrombin III and deactivated motility toward chemokines [11]. Moreover, the absence or blocking of SDC-4 signaling in ovalbumin-sensitized mice resulted in a reduced asthma phenotype compared with control animals [14]. Therefore, we hypothesized that SDC-4 could possibly regulate allergic inflammation, such as atopic dermatitis (AD). AD is a major pruritic inflammatory skin disease, related to Th2-driven

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allergic immune responses. Scratching behavior from intensive itching causes skin barrier dysfunction and exacerbation of AD [4]. In this study, we examined SDC-4 expression in sera and skin of AD patients.

Materials and methods

Patients and samples

Blood samples were collected from 59 AD patients (mean $\pm$ standard deviation age, 32.1 ± 12.56 years; 32 men and 27 women) and 56 healthy control subjects (33.8 ± 13.13 years; 29 men and 27 women). Skin samples were collected from ten patients with AD and ten healthy control subjects. All AD patients were given diagnoses according to the criteria of Hanifin and Rajka (1980). The disease severity was determined by Physician Global Assessment and the Eczema Area and Severity Index (EASI). We rated itch by the visual analogue scale (VAS) 0–10, asking the patients to mark a point on the line corresponding to mean itch during the last 7 days before blood draw. Most patients had not been treated with topical corticosteroids or topical calcineurin inhibitors for more than 1 month when blood samples were collected. Some used topical treatment, but the skin diseases were not fully controlled. The 56 healthy controls had no history of allergic diseases, such as AD, allergic rhinitis, or asthma. Both AD patients and healthy controls were all Japanese. All samples were obtained after informed consent. The Medical Ethical Committee of the University of Tokyo approved all the described studies, and the study was conducted according to the Declaration of Helsinki Principles.

Enzyme-linked immunosorbent assay

Quantification of serum SDC-4 levels was performed using a Human Syndecan-4 DuoSet ELISA kit (R&D systems, Minneapolis, MN, USA). Optical densities were measured at 450 nm using a Bio-Rad Model 550 microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). The measured values from individual patients were plotted by dots.

Immunohistochemistry

We performed immunohistochemical staining for SDC-4 on AD lesional skin ($n = 10$) and normal skin ($n = 10$). The samples were taken from the skin lesion that was most severe. The biopsy specimens were taken about 2 cm in diameter, deeply enough to reach subcutaneous tissue. Briefly, 5 μ m-thick tissue sections from formaldehyde-fixed and paraffin-embedded samples were dewaxed and rehydrated. Rabbit anti-human SDC-4 antibody (Immuno-

Biological Laboratories, Japan) or control rabbit IgG (Vector Lab, Burlingame, CA, USA) was applied, followed by ABC staining (Vector Lab, Burlingame, CA, USA). Diaminobenzidine was used for visualizing the staining, according to the manufacturers' instructions.

Real-time reverse-transcriptase PCR

Messenger RNA (mRNA) was obtained from AD lesional skin ($n = 8$) and normal skin ($n = 8$). All skin samples were snap frozen in liquid nitrogen and stored at -80°C . Total RNA was isolated from frozen tissue using RNeasy fibrous tissue mini kit (QIAGEN, Velno, The Netherlands) and complementary DNA was synthesized using TaqMan[®] Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA). The expression levels of SDC-4 mRNA were analyzed by a real-time PCR quantification method according to the manufacturer's instructions (Applied Biosystems). Gene expression was quantified using the TaqMan gene expression assay (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize the mRNA. Primers for SDC-4 and GAPDH were obtained from Applied Biosystems. All samples were analyzed in parallel for GAPDH gene expression as an internal control. The relative expression levels of each gene were determined by the $2^{-\Delta\Delta C_T}$ method.

Statistical analysis

Statistical analysis was carried out with the Mann–Whitney's U test to compare the distribution of two unmatched groups. One-way ANOVA followed by Tukey post-hoc test for Kruskal–Wallis test and Scheffe's F test was used for multiple comparisons. Spearman's rank correlation coefficient was used to evaluate the correlation with clinical data. Wilcoxon matched-pairs signed-rank test was used for comparison before and after treatment. p values of <0.05 were considered statistically significant.

Results

Elevated serum SDC-4 levels in AD

Serum SDC-4 levels in AD patients were significantly higher than those in healthy individuals (1406.6 ± 1062.3 and 711.8 ± 671.2 pg/ml, respectively; $p < 0.01$) (Fig. 1a). We also analyzed serum SDC-4 levels according to the severity of AD. Serum SDC-4 levels were higher as the disease severity increased and reached a significant difference between moderate and severe AD patients as well as between mild and severe AD patients (Fig. 1b). Importantly, serum SDC-4 levels positively correlated with EASI, a widely used

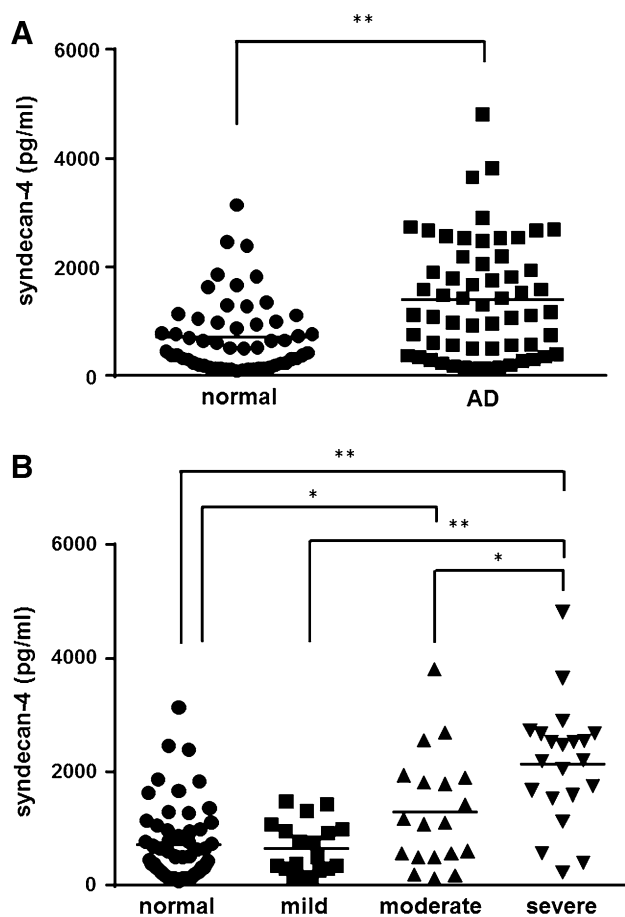


Fig. 1 Serum syndecan-4 levels in atopic dermatitis (AD) and healthy controls. Serum syndecan-4 levels in 59 AD patients and 56 healthy individuals. Each bar indicates the mean of the group (a). Serum syndecan-4 levels in AD patients according to the disease severity (b). * $p < 0.05$ and ** $p < 0.01$

quantitative evaluation of AD severity ($r = 0.57$, $p < 0.01$) (Fig. 2a), and VAS itch scores ($r = 0.61$, $p < 0.01$) (Fig. 2b). There was no significant correlation between serum SDC-4 levels and serum IgE or CCL17 levels (data not shown). Furthermore, we compared serum SDC-4 levels in 17 AD patients before and after treatment. They were treated with topical corticosteroids, topical calcineurin inhibitors, and/or oral antihistamines. They were also treated with white petrolatum, urea ointment, heparinoid cream, or other moisturizers. The duration of therapy ranged from a week to a year. Serum SDC-4 levels significantly decreased after treatment ($p < 0.05$) (Fig. 2c).

Enhanced SDC-4 expression in AD lesional skin

We next assessed SDC-4 mRNA expression in AD lesional skin and in normal skin by quantitative RT-PCR. SDC-4

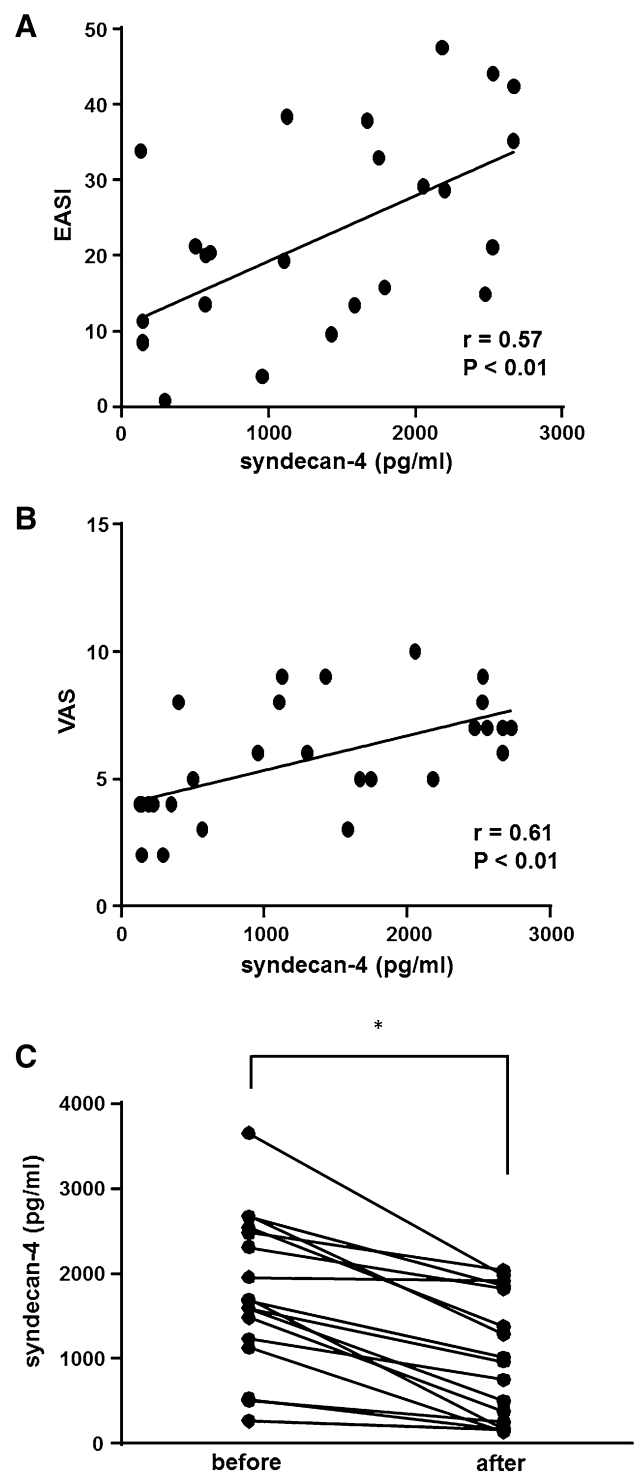
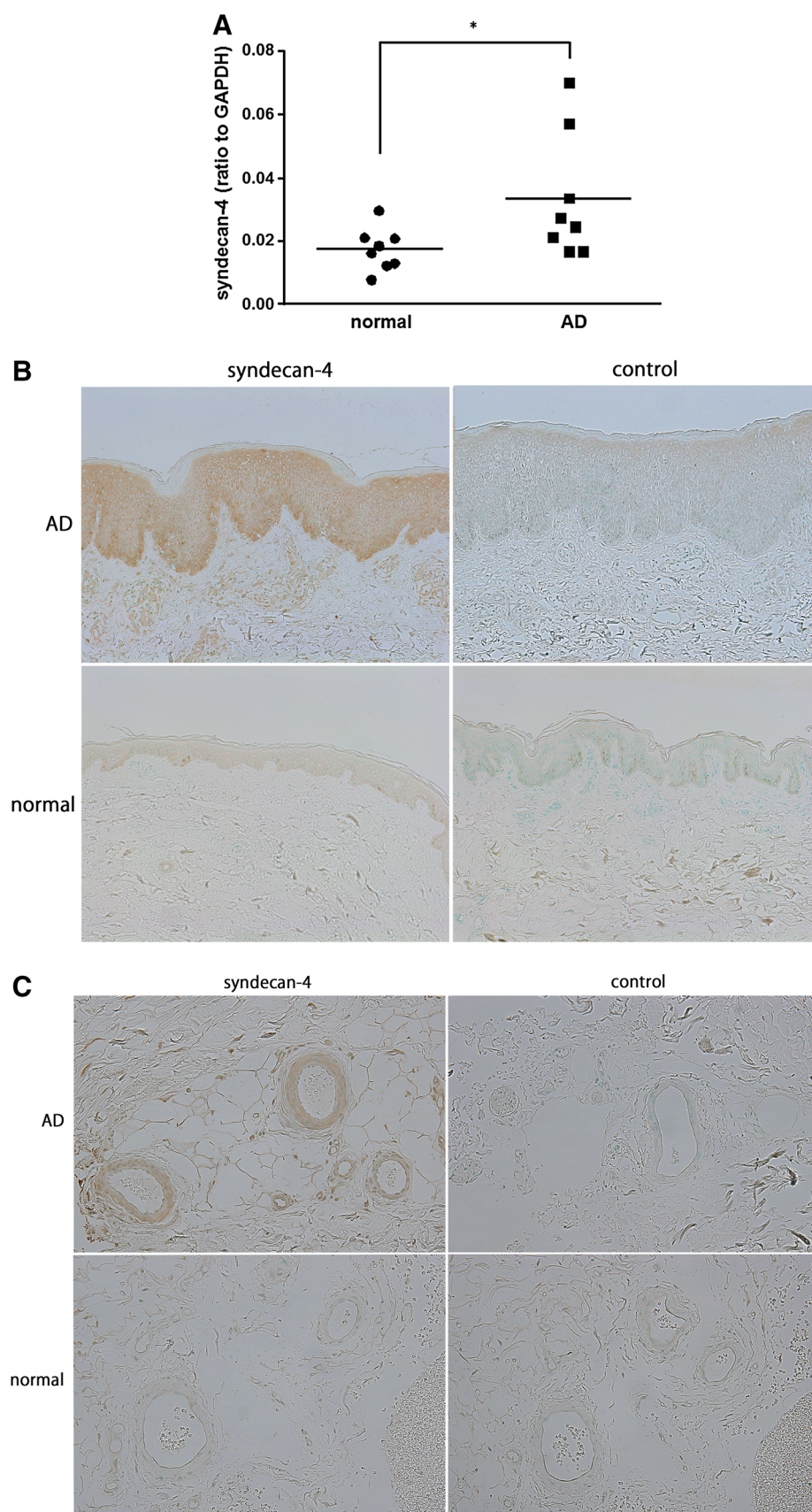


Fig. 2 Correlations between serum syndecan-4 levels and the disease markers of atopic dermatitis (AD). Serum syndecan-4 levels significantly correlated with Eczema Area and Severity Index (EASI) (a). Serum syndecan-4 levels significantly correlated with visual analogue scale (VAS) itch scores (b). Serum syndecan-4 levels decreased after treatment (c). * $p < 0.05$

Fig. 3 Syndecan-4 mRNA expression levels of lesional skin of AD were significantly higher than those of normal skin (a). Immunohistochemistry for syndecan-4 and isotype control using AD lesional skin and normal skin (b, c). Representative pictures from each group (b $\times 100$, c $\times 200$). $*p < 0.05$



mRNA expression levels in AD skin were significantly higher than those in normal skin ($p < 0.01$) (Fig. 3a). Immunohistochemical staining revealed that SDC-4 was mainly expressed in the epidermis and blood endothelial cells (Fig. 3b, c). The expression in the epidermis and endothelial cells in AD lesional skin was much higher than that in healthy skin (Fig. 3b, c).

Discussion

Our study revealed that SDC-4 expression in sera and skin was significantly enhanced in AD patients compared with normal controls. Importantly, serum SDC-4 levels correlated with disease severity and VAS itch scores.

SDC-4 participates in the migration of dendritic cells (DCs) [6] and acts as an accessory molecule during T-cell activation [1]. Allergic immune response begins with signals delivered from antigen-presenting cells, such as DCs, which capture and process antigens and migrate to lymphoid organs [3]. In AD, the persistent stimulation of epidermal DCs (Langerhans cells) through a defective skin barrier could result in chronic Th2-driven allergic inflammation [10]. It was recently demonstrated that a disturbed SDC-4 signaling caused a diminished chemokine receptor CCR7 expression on DCs and a reduced directional DC migration, resulting in an attenuated asthma phenotype in SDC-4-deficient mice. Most importantly, even established asthma is significantly decreased using anti-SDC4 antibody [14]. Furthermore, the ligation of SDC-4 with anti-thrombin III induced eosinophil migration, suggesting that signaling via SDC-4 might control human eosinophil locomotion [11]. It was recently reported that syndecan-4 therapy for mouse ischemic hind limbs induced a marked immunomodulation in the tissues, increasing the polarization of macrophages toward the M2 phenotype [7]. Taken together, SDC-4 plays important roles in Th2-driven immune responses, which is also supported by our results with AD samples.

Many reports have suggested that SDC-4 is involved in angiogenesis [7, 8, 13, 16]. Cultured human umbilical vein endothelial cells expressed SDC-3 and SDC-4, and lipopolysaccharide and interleukin 1 β significantly increased SDC-4 expression [16]. In skin, epidermis, blood endothelial cells, fibroblasts, and inflammatory cells are reported to express SDC-4 [5], which is consistent with our results. Enhanced SDC-4 expression in blood endothelial cells may be important for the migration of inflammatory cells in AD lesional skin.

In summary, we demonstrated that the SDC-4 expression increased in sera and skin in patients with AD. Our results suggested that SDC-4-dependent signaling might

contribute to the development of AD. SDC-4 can be a therapeutic target in AD, as was reported in a mouse asthma model [14].

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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Ethical approval The medical ethical committee of the University of Tokyo approved all described studies, and the study was conducted according to the Declaration of Helsinki Principles.

Informed consent Informed consent was obtained from all individual participants included in the study.

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