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ORIGINAL PAPER

Podoplanin suppresses the cell adhesion of epidermal keratinocytes via functional regulation of β1-integrin

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Abstract

Epidermal stem cells adhere more efficiently to the extracellular matrix (ECM) than the less adhesive differentiating cells due to their high expression of cell adhesion molecules including β1-integrin. Podoplanin is majorly expressed in the markedly proliferative and differentiating basal cells of the wounded and psoriatic epidermis. This study was designed to reveal podoplanin's function in human epidermal keratinocytes (HEK) focusing on its interaction with β1-integrin. We analyzed the adhesion and differentiation of HEK in both podoplanin-overexpressing and -knock-down cells, considering their β1-integrin levels. The basal layer of IL-22-treated hyperproliferative reconstituted epidermis cells (simulating basal hyperproliferative psoriatic epidermal basal cells) expressed higher podoplanin levels than the untreated control cells. The adhesiveness of HaCaT cells, which do not express podoplanin, was reduced after the overexpression of podoplanin. HEK with podoplanin overexpression suppressed the cell adhesion to type I collagen (while downregulating β1-integrin functions) and podoplanin silencing augmented it (by increasing active ECM-bound β1-integrin). The increased cell adhesion to type I collagen induced by podoplanin silencing could be reversed by addition of P5D2, a neutralizing antibody against β1-integrin. In the psoriatic epidermis, podoplanin expression was especially upregulated on the rete ridges of the basal cell layer. This expression pattern was inversely correlated with the total/ECM-bound active β1-integrin-expression, which was stronger at the basal cell layer covering the dermal papillae. Our results indicate that podoplanin inhibits the cell ECM attachment by suppressing β1-integrin and initiating HEK differentiation. Podoplanin is presumably involved in the pathogenesis of psoriasis.

Keywords Psoriasis · Epidermis · Differentiation · Stem cell · Therapeutic target

Introduction

Psoriasis is a chronic and persistent inflammatory skin disorder characterized by reddish scaly plaques on mechanically stressed areas such as the extensor sides of extremities and lower back [22]. A Th17-oriented cytokine-network is involved in its pathogenesis, as demonstrated by the excellent therapeutic effect of biologics targeting Th17-related cytokines [25]. A fully developed psoriatic outbreak reveals distinctive histopathological features with hyperproliferation of epidermal keratinocytes and inflammation set on both the epidermal and dermal layers [3]. The turnover time in the psoriatic epidermis is shortened by one-seventh of that in the normal epidermis [33], and a distinctive epidermal structure results from dermal papillae plasticity changes due to the intercellular pressure exerted by the proliferative epidermal keratinocytes [12]. While the epidermal proliferative unit model [1] and the committed progenitor model [13] have been proposed as mechanisms maintaining the stratified epithelial structure, the proliferating basal keratinocytes' need to supply differentiating keratinocytes is possibly the product of accelerated asymmetrical cell divisions induced by IL17A, a key cytokine in the pathogenesis of psoriasis [4]. Adhesion molecules binding to the extracellular matrix (ECM) work to reserve the stem cell population and β1-integrin is one of the most important factors [32]. Wounded and psoriatic epidermal cells express less β1-integrin and, thus, less adhesive cells are produced maintaining the balance of cell proliferation and desquamation of the cornified cells [27].

Podoplanin is a transmembranous glycoprotein associated with lymphangiogenesis, platelet aggregation, cell migration, and oncogenesis [29]. Advances in the basic

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and clinical research on oncology suggest the possibilities of podoplanin-targeting therapies against several malignant tumors, such as brain tumor and melanoma [24]. A polymorphism of the podoplanin gene located at p36.21 of chromosome 1 has been strongly associated with intrinsic atopic dermatitis, one of the major inflammatory skin disorders [21]. Podoplanin expression is markedly induced at the basal cell layer of the re-epithelizing wounded epidermis and hyperproliferative agranular psoriatic epidermis [11], in contrast to the minimal expression in the normal interfollicular epidermal cells. Cytokines relating to the pathogenesis of psoriasis, IFN-γ and IL-22 [16], stimulate podoplanin expression in a STAT-3-dependent manner [11]. Podoplanin is crucial in the excretion of IL-17 from activated peripheral blood mononuclear cells (PBMC) via the interaction between podoplanin-expressing PBMC and dermal mesenchymal cells [23]. The specific induction of podoplanin and the excretion mechanism of IL-17 may play a crucial role in the pathophysiology of psoriasis, a multifactorial disorder simulating the wound healing process [17].

In this study, we present evidence that podoplanin downregulates the attachment of normal human epidermal keratinocytes (HEK) to the ECM by suppressing integrin functions. This was associated with the initiation of HEK differentiation in vitro and may be involved in the remodeling process of the hyperproliferative psoriatic epidermis [12], which is characterized by highly accelerated balanced proliferation and differentiation of cells.

Methods

Cell culture

Adult HEK (#00192627, Lonza, Basel, Switzerland) were cultured in KBM-Gold serum-free medium #00192060, Lonza. HEK293 cells [20] and HaCaT cells [15] were maintained in Dulbecco's modified Eagle's medium (DMEM; SIGMA, St. Louis, MO, USA) containing 10% fetal calf serum. LabCyte Epi-Model 6D (#40112E6, J-TEC, Gamagori, Aichi, Japan), the reconstituted epidermis cultured for 6 days, was fed every other day with supplied culture medium (J-TEC) containing 50 ng/ml IL-22 or IL-24 (Peprotech, Rocky Hill, NJ, USA) and collected after the culture with cytokines for 8 days.

Tissue sections and immunostaining

We obtained consents from patients with psoriasis to use frozen formalin-fixed and paraffin-embedded tissue sections under the approval of the ethical committee of the Asahikawa Medical University. Fresh frozen sections were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS), and deparaffinized sections were boiled in 10 mM citrate buffer for antigen retrieval. Furthermore, we blocked these sections with 5% BSA in PBS for 1 h and incubated them with primary antibodies diluted in 5% BSA in PBS overnight at 4 °C. We employed Alexa fluor-conjugated secondary antibodies (Invitrogen, Carlsbad, CA) or the avidin–biotin complex method for visualization. We stained nuclei with Hoechst 33342 dye (Invitrogen) and observed the immunofluorescent-stained sections using a fluorescent microscope system (Olympus, Tokyo, JAPAN).

Antibodies and reagents

We used mouse monoclonal antibody, D2-40 (DAKO), and rat monoclonal antibody, NZ-1 (AngioBio, Del Mar, CA, USA), for podoplanin detection. Additionally, anti-β-tubulin, (Sigma, St. Louis, MO, USA), anti-β1-integrin (BD bioscience, Franklin Lakes, NJ, USA), and anti-involucrin (Biomedical Technologies Inc., Stoughton, MA, USA) rabbit polyclonal antibodies were employed for the detection of each corresponding molecule. We used mouse monoclonal HUTS21 antibody (BD bioscience) to identify the extracellular matrix-bound β1-integrin and mouse monoclonal antibody, M2 (Sigma), to detect the exogenous Flag-tag.

Western blotting

We washed the cells twice with ice-cold PBS and lysed them in ice-cold RIPA buffer containing a protease inhibitor cocktail (Roche, Indianapolis, IN), as described [9]. Protein concentrations were determined with the BCA protein assay kit (Pierce, Rockford, IL, USA). We separated the proteins (20–50 μg per lane) using SDS-PAGE and transferred them to Hybond-P nitrocellulose membranes (Amersham Bioscience, Piscataway, NJ). Blotted membranes were blocked with tris-buffered saline containing 0.1% Tween-20 and 5% skimmed milk and were then incubated with primary antibodies overnight at 4 °C. We visualized the proteins with anti-mouse or anti-rabbit IgG horseradish-peroxidase—linked antibodies (Amersham Bioscience) for 1 h, followed by chemiluminescence detection (ECL plus, Amersham Bioscience).

Immunoprecipitation

For precipitation of ECM-bound activated β1-integrin and total β1-integrin, we incubated the cell lysates with 0.5 μg/ml of HUTS21 or anti-total β1-integrin antibodies (BD bioscience) at 4 °C overnight, and then precipitated the immune complexes with protein G-conjugated agarose-beads (SantaCruz Biotech, Dallas, TX, USA).

Adenovirus vectors

Flag-tagged human podoplaninc DNA obtained by RT-PCR was subcloned into the *Swa*I site of the cosmid vector, pAxCAwt. Oligomer pairs coding human podoplanin-specific shRNA, shPDPN1 (5′-GATCCGGACCATTGGATCGATATTCTGTGAAGCCACAGATGGGAATATCGATCCAATGGTCCTTTTTTA-3′ and 5′-AGCTTAAAAAAGGACCATTGGATCGATATTCCCATCTGTGGCTTCACAGAATATCGATCCAATGGTCCG-3′) and shPDPN2 (5′-GATCCGGAAGTCGATAGTCTCAAACTGTGAAGCCACAGATGGGTTTGAGACTATCGACTTCCTTTTTTA-3′ and 5′-AGCTTAAAAAAGGAAGTCGATAGTCTCAAACCCATCTGTGGCTTCACAGTTTGAGACTATCGACTTCCG-3′) were annealed and subcloned into *BamH*I and *Hind*III sites of the pBAsi-hU6 vector (TAKARA, Otsu, Japan). We subcloned the hU6 RNA polymerase promoter-driven shRNA and the empty constructs into the pAxcwit2 promoter-less cosmid vector. The adenoviral vectors were generated using the COS-TPC method in HEK293 cells (TAKARA) [20]. We named the adenoviral vectors carrying the empty construct as Ax-shcont. AxLacZ carrying β-galactosidase [20] and Axpodoplanin [11] were employed for the control vector and podoplanin overexpression, respectively. Both Ax-shpodoplanin1 [7] and Ax-shpodoplanin2 were used for silencing of endogenous podoplanin expression. These adenoviral vectors were amplified in HEK293 cells and titered according to the manufacturer's protocol. The indicated multiplicity of infections (MOIs) of each adenoviral vector was infected into the HEK for 1 h, and fresh culture medium was added to the cells.

Adhesion assays

We suspended 10^5 cells in 100 µl of culture medium, seeded them into collagen-I-coated 24 well plates and incubated them for the indicated times. Moreover, we rinsed each well twice with PBS and added fresh culture medium containing cell counting Kit solution (#CK04, DOJINDO, Mashiki, JAPAN) in one-tenth volume of the medium. After 1 h incubation, we measured the absorbance of medium in each well at 450 nm. We preincubated the keratinocytes with mouse monoclonal anti-β1-integrin neutralizing antibody, P5D2 (20 µg/ml; R&D, Minneapolis, MN) to neutralize the β1-integrin-mediated cell adhesion. We repeated each experiment more than thrice and analyzed the results statistically using Student's *t* test.

Co-culture of podoplanin-overexpressing and control keratinocytes

We mixed equal counts of HEK, independently infected with AxLacZ and AxPDPN, at MOI of 20, and then seeded 10^6 cells on collagen-coated cover slips in a ϕ35 mm dish. We collected the cover slips at overconfluent culture conditions and used them for immunostaining after 4% paraformaldehyde fixation.

Results

Podoplanin is majorly expressed in the conventionally cultured HEK and in IL-22-treated reconstituted epidermis simulating the hyperproliferating psoriatic epidermis

The podoplanin expression of HEK cultured in the conventional low calcium medium was considerably higher than that of the reconstituted 3-dimensional culture (Fig. 1a). Both IL-22 (50 ng/ml) and IL-24 (50 ng/ml) treatments

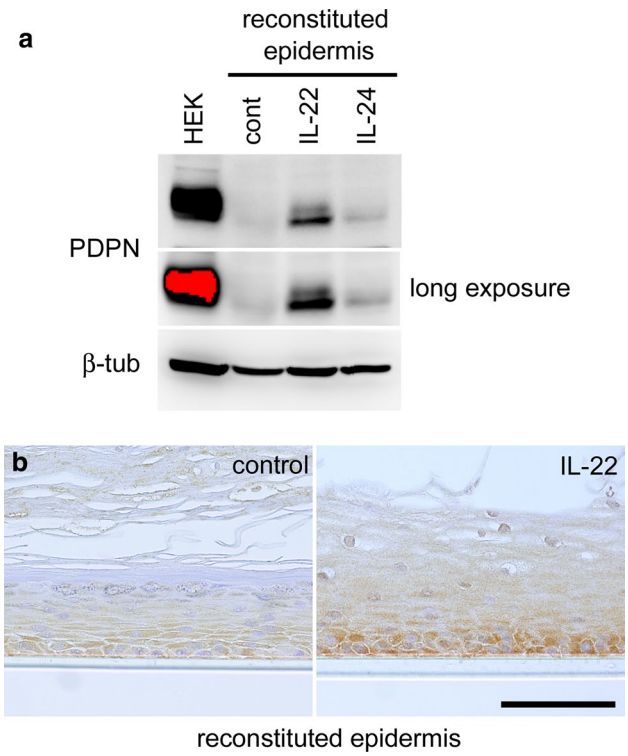

Fig. 1 IL-22-dependent induction of podoplanin (PDPN) expression in stratified reconstituted epidermis. **a** Western blotting for podoplanin in the conventionally cultured human epidermal keratinocytes (HEK) and stratified reconstituted epidermis. β-Tubulin (β-tub) was employed as an internal control. Podoplanin expression in the conventionally cultured HEK was much stronger than that of the reconstituted epidermal cells. Podoplanin expression was significantly induced by IL-22-treatment (IL-22) and was less so by IL-24-treatment. **b** Immunohistochemistry for podoplanin in the reconstituted epidermis. IL-22 treatment (IL-22) significantly induced the podoplanin expression at the basal cell layer of stratified reconstituted epidermis compared with the control epidermis (control). Bar 100 µm

induced podoplanin expression in the reconstituted epidermis; however, the expression level was higher after IL-22 treatment than after IL-24 treatment (Fig. 1a). The IL-22-treated reconstituted epidermis presents features resembling the hyperproliferating psoriatic epidermis, such as a hyperkeratotic thickened epidermis with parakeratosis but without granular layers [10] (Fig. 1b). Podoplanin expression was majorly induced at the basal cell layer of the IL-22-treated reconstituted epidermis in contrast to that of the untreated epidermis control (Fig. 1b).

Podoplanin overexpression suppresses the cell adhesion to type I collagen in HaCaT keratinocytes, which do not normally express podoplanin

As the basal keratinocytes form adhesion structures (hemidesmosomes and adherens junctions) to the ECM [6], we hypothesized that podoplanin might regulate the keratinocyte adhesion to the ECM. In contrast to the strong expression we observed in HEK, we did not detect podoplanin expression in HaCaT (an immortalized human keratinocyte cell-line; Fig. 2a). Therefore, to analyze the function of podoplanin in the keratinocytes, we overexpressed podoplanin in HaCaT cells. The expression was strongly induced by the AxPDPN-infection (in a dose-dependent manner; Fig. 2b). In the podoplanin-overexpressing HaCaT cells, cell adhesion to type I collagen was decreased by one-half to a third of that in control cells (β-gal) at 10 min (Fig. 2c).

Podoplanin downregulation associates with the adhesiveness of HEK to collagen I-coated plates

Adenovirus vectors carrying shRNA constructs against endogenous podoplanin expression, AxshPDPN1 (shPDPN1) and AxshPDPN2 (shPDPN2), successfully suppressed the endogenous podoplanin expression in HEK (Fig. 3a, c). In podoplanin-silenced HEK (shPDPN1 or shPDPN2), the cell adhesion to the type I collagen-coated culture plate was significantly increased up to approximately twice that of the control keratinocytes (shcont; $p < 0.01$); refer Fig. 3b, d. In contrast, as observed in the HaCaT cells, the podoplanin overexpression reduced the cell adhesion to type I collagen-coated culture plates (data not shown).

Podoplanin regulates the ECM-bound β1-integrin level but not the total β1-integrin expression level in HEK

Considering that β1-integrin is essential for the adhesion of epidermal keratinocytes to collagen-I [31], we examined whether β1-integrinis are involved in the podoplanin-mediated regulation of cell adhesion. Neither silencing nor

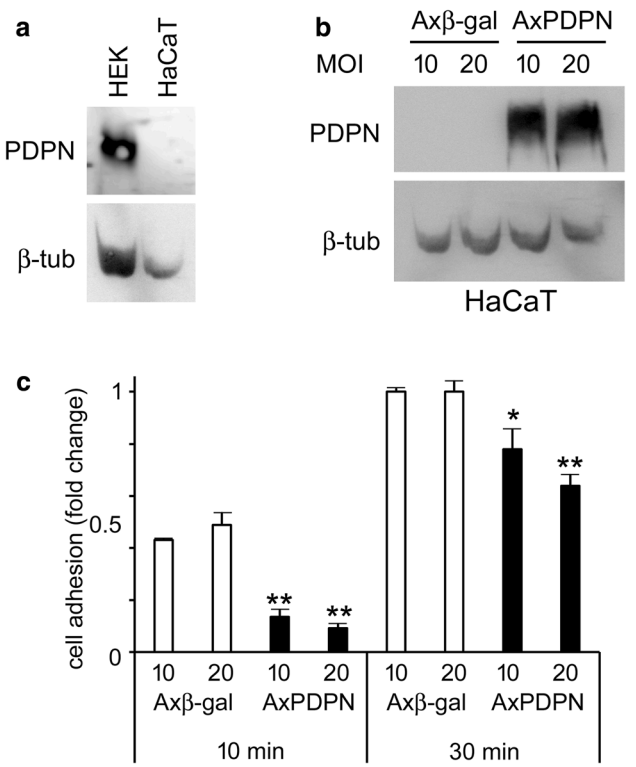

Fig. 2 Podoplanin-induced suppression of cell adhesion of HaCaT cells, an immortalized epidermal keratinocyte cell-line, to collagen I-coated plate. **a** Podoplanin expression was not detected in HaCaT cells in contrast to the strong expression in HEK. **b** Podoplanin expression was induced in a manner dependent on the efficacy of infection by an adenoviral vector carrying a flag-tagged human podoplanin cDNA construct (AxPDPN) when compared with the expression in cells infected with a control vector (Axβ-gal). **c** Podoplanin reduced HaCaT cell adhesion to collagen I-coated plate (mean value ± standard error [SE]). Adhered cells were counted using an MTT assay at 10 and 30 min following the cell seeding. The data of control (Axβ-gal) at 30 min were used as a reference and compared with the podoplanin-overexpresing HaCaT (AxPDPN). Numbers at the horizontal axis indicate multiplicity of infection (MOI) of each vector. $*p < 0.05$, $**p < 0.01$ compared with the control (Axβ-gal)

overexpression of podoplanin affected the total endogenous β1-integrin expression level (Fig. 4a, c). Expression of α3-integrin, one of the partners binding β1-integrin, was not affected by podoplanin expression either (data not shown). In contrast, the expression level of the ECM-bound β1-integrin was influenced by the podoplanin expression. The active β1-integrin was immunoprecipitated by HUTS21 antibody [27] and detected by anti-pan-β1-integrin antibody. ECM-bound β1-integrin was increased up to 1.7 times the level of control keratinocytes (shcont) after podoplanin silencing (shPDPN); however, it decreased by podoplanin overexpression (Fig. 4a, c). The upregulated cell adhesion induced by podoplanin silencing was completely abolished by administration of the neutralizing antibody against β1-integrin, P5D2 (Fig. 4b).

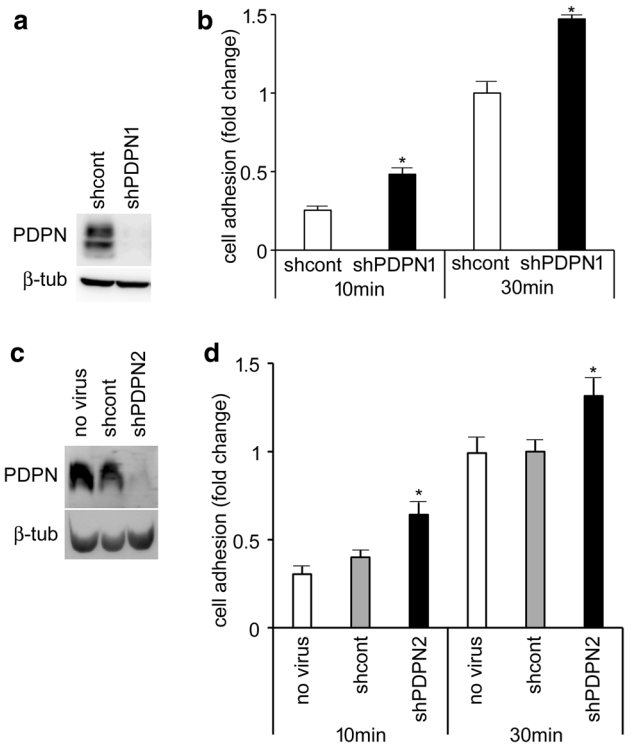

Fig. 3 Podoplanin-specific silencing by independent shRNA constructs strengthens cell adhesion of HEK to collagen I-coated plate. **a** Endogenous podoplanin expression was significantly reduced by an adenovirus vector carrying a podoplanin-specific shRNA construct, shPDPN1. *shcont* control adenovirus vector. **b** Podoplanin-specific silencing using shPDPN1 strengthens HEK cell adhesion to the collagen I-coated plate. The data of control (shcont) at 30 min were used as a reference. $*p < 0.01$, compared with the control. **c** Endogenous podoplanin expression was significantly reduced by another independent adenovirus vector carrying a podoplanin-specific shRNA construct, shPDPN2. **d** Podoplanin-specific silencing using shPDPN2 also strengthens the cell adhesion of HEK to the collagen I-coated plate. The data from the control (shcont) at 30 min were used as a reference. $*p < 0.01$, compared with the control

Podoplanin overexpression in HEK is correlated with induction of involucrin, a differentiation marker of the epidermal keratinocytes

Furthermore, we investigated whether podoplanin would affect the keratinocytes' differentiation. To mimic the keratinocyte conditions similar to those of the epidermal basal cell layer, we co-cultured the podoplanin-overexpressing keratinocytes and control keratinocytes on collagen-I-coated cover slips. Under these conditions, the less adhesive cells should enter the differentiation process and express differentiation markers, such as involucrin [31]. As expected, the involucrin positive cell ratio of podoplanin-expressing (Flag +) HEK was 2.5 times higher than that of the control (Flag −) HEK, at the overconfluent mixed culture conditions (Fig. 5a, b).

Inverse correlation between podoplanin and β1-integrin expressions in the hyperproliferative psoriatic epidermis

Podoplanin is expressed at the basal cell layer of the psoriatic hyperproliferative epidermis, in contrast to the podoplanin-negative normal interfollicular epidermis [11]. The podoplanin expression is strong at the rete ridges. In contrast, the podoplanin expression is low at the basal cells covering the tips of the dermal papillae. We observed an inverse correlation between the podoplanin- and β1-integrin-expressions in the psoriatic hyperproliferative epidermis (Fig. 6a). While the expression of β1-integrin was higher at the basal cell layer covering the dermal papillae, the β1-integrin-expression was lower at the basal cell layer of rete ridges (Fig. 6a). We also observed an inverse correlation between the expressions of podoplanin and ECM-bound active β1-integrin in the hyperproliferative psoriatic epidermis (Fig. 6b). The expression of ECM-bound active β1-integrin (HUTS21) was higher at the basal cell layer covering the dermal papillae compared with that at the basal cells of rete ridges where podoplanin expression was stronger (Fig. 6b).

Discussion

Based on our results, we propose a novel regulatory mechanism of ECM cell adhesion via suppression of ECM-bound β1-integrin by podoplanin. We were able to reproduce the inverse correlation between the expressions of podoplanin and ECM-bound β1-integrin using hyperproliferative psoriatic epidermis, suggesting that podoplanin is involved in the pathogenesis of the psoriatic epidermal architecture.

Podoplanin, which is not expressed in the normal interfollicular epidermis, is strongly expressed at basal cell layers of wounded or psoriatic epidermis, which reveal markedly expanded proliferating cell populations [11]. The podoplanin expression depends on the STAT-3 signaling pathway that is activated in both wounded and psoriatic hyperproliferative epidermis [11]. Even under conventional culture conditions, the normal human keratinocytes express podoplanin constitutively, suggesting that the cultured keratinocytes are at hyperproliferative conditions simulating the fully developed psoriatic epidermis, but not the normal interfollicular epidermis. Similarly, the enzymatic dissociation of the epidermis can induce an injury-associated molecular response in the keratinocytes [2]. In IL-22-treated hyperproliferative reconstituted epidermis simulating psoriasis, the podoplanin expression was strongly induced at the basal cell layer compared with the expression in the control reconstituted epidermis (Fig. 1a, b), suggesting a STAT3-dependent mechanism of podoplanin expression in the stratified epidermal keratinocytes. While the detailed mechanism remains

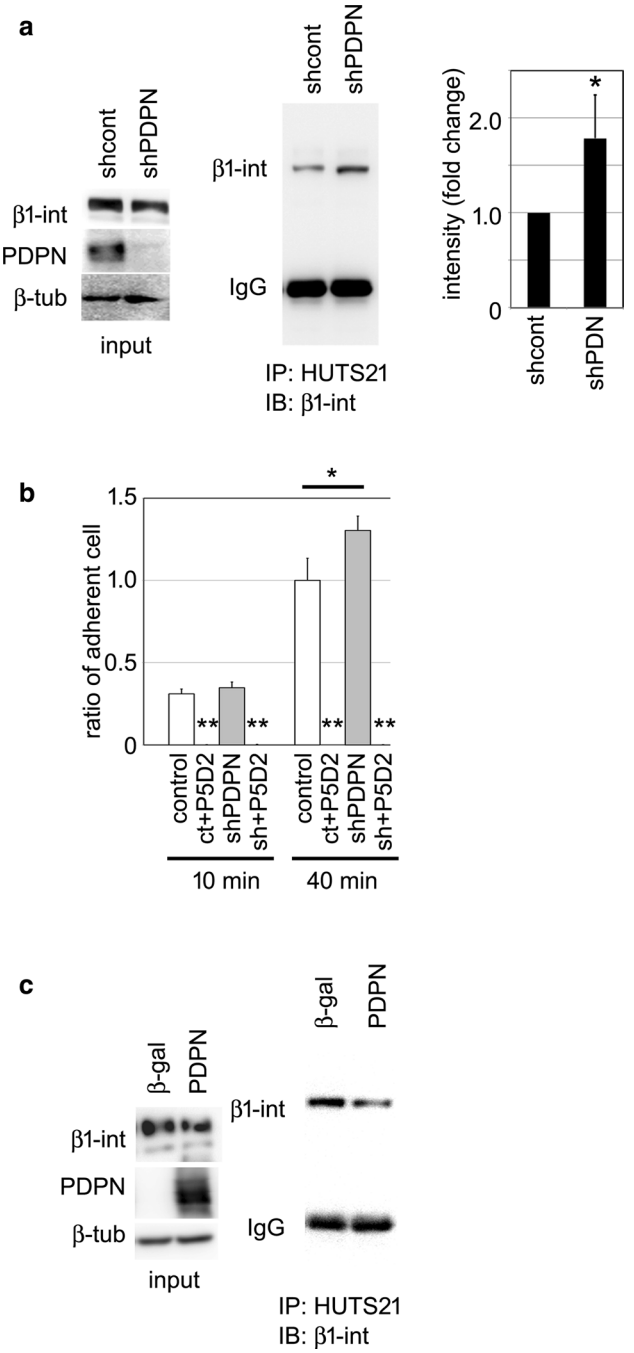

Fig. 4 Podoplanin alters the level of ECM-bound active form but not that of total β1-integrin. **a** Podoplanin silencing increased the ECM-bound active form of β1-integrin in HEK. While podoplanin silencing did not change the total β1-integrin expression (shPDPN; left: input), the ECM-bound active form was increased up to 1.7 times (graph). The ECM-bound active form of β1-integrin was immunoprecipitated by a specific antibody, HUTS21. Furthermore, an antibody recognizing total β1-integrin was used to detect the precipitated active β1-integrin. The experiment was independently repeated thrice. $*p < 0.01$ compared with control. **b** The podoplanin-specific silencing-induced cell adhesiveness of HEK was completely abolished by the β1-integrin-specific neutralizing antibody, P5D2. Cell adhesiveness was increased by podoplanin-specific silencing (shPDPN) at 40 min ($*p < 0.05$). Neutralization of β1-integrin by P5D2 completely abolished the cell adhesion of HEK ($**p < 0.01$). **c** Podoplanin overexpression inhibited the ECM-bound active β1-integrin. While HEK was infected with AxPDPN at MOI 2, which did not affect the β1-integrin expression level (left: input), ECM-bound β1-integrin was decreased (right)

unclear, the basal cell-limited induction of podoplanin in the hyperproliferative-stratified epidermis suggests an association of podoplanin to the ECM cell adhesion molecules of the basal cells. While apparent differences in the podoplanin expression were not detected in the microarray analyses using lesional and nonlesional psoriatic skin [8], the limited expression of podoplanin to the basal cells in psoriatic epidermis [10] and possible contamination by hair follicles may explain these results. Reports suggest that the asymmetrical cell division can be an essential mechanism for the formation of the psoriatic hyperproliferative epidermis [4]. In the imiquimod-induced psoriasiform dermatitis model, IL17A, a crucial cytokine in the pathogenesis of psoriasis, is involved in the accelerated asymmetrical cell division of basal keratinocytes. These molecular mechanisms can cooperatively be involved in the pathological mechanisms resulting in the psoriatic epidermal architecture.

As discussed in the various congenital and acquired blistering disorders, basal keratinocytes attach firmly to the ECM via hemidesmosome and adherens junctions that are configured by various adhesive molecules, such as laminin, type XVII collagen, and integrins. Among them, the integrin family is crucial for cell attachment to the ECM, and β1-integrin is a well-known marker of the epidermal stem cells [14]. β1-integrin adheres to the type I collagen and fibronectin via α2- and α3/5-integrin subunits, respectively [31]. We observed the total and ECM-bound β1-integrin-expressions in basal cells over the tips of dermal papillae, consistent with a report [26]. While adhesion molecules are indispensable for maintaining the epidermal stem cells, the adhesiveness needs to be regulated to produce migrating, proliferating and differentiating populations of the epidermal cells during wound healing and similar processes (like psoriasis). For example, β4-integrin phosphorylation, a downstream consequence of growth factor signaling, such as the one initiated by epidermal growth factor, accelerates endocytosis of β4-integrin and results in the disassembly of hemidesmosomes [18]. The expression of β1-integrin is downregulated in the differentiating cell populations [31] (Fig. 6). Here, we reported that podoplanin suppresses the cell adhesion of keratinocytes through inhibition of ECM-bound β1-integrin. The inverse expression patterns of podoplanin and ECM-bound β1-integrin in the hyperproliferative psoriatic epidermis also suggest the involvement of podoplanin in the mechanism of epidermal stem cell transition to differentiating cells via inhibition of β1-integrin function. While the molecular mechanism of podoplanin-mediated

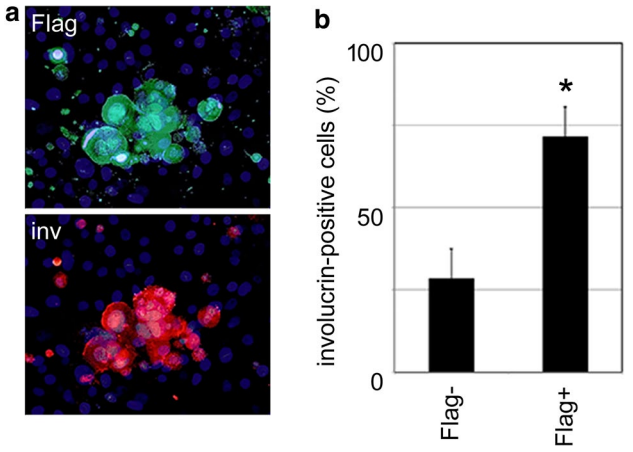

Fig. 5 Podoplanin-overexpression induced HEK differentiation. **a** More marked involucrin positive cells (inv+) were detected in the podoplanin-overexpressing HEK (Flag +) than in the control HEK (Flag −) under overconfluent conditions. **b** Inv + cells were observed 2.5 times more than the Flag-control cells in Flag-positive podoplanin-overexpressing HEK (*$p < 0.01$)

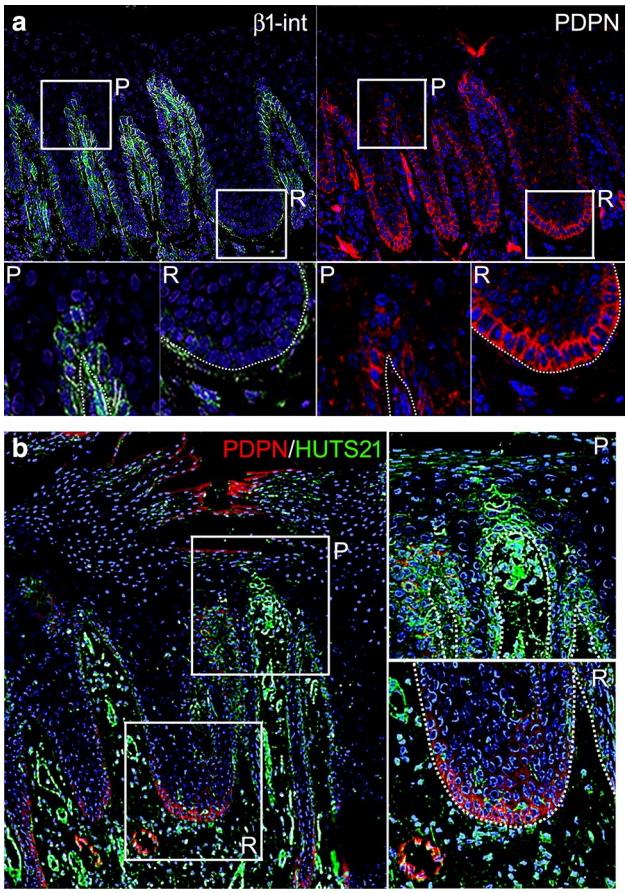

Fig. 6 Inverse expression of podoplanin and β1-integrin in psoriatic hyperproliferative epidermis. **a** β1-Integrin (green) is marked at the basal cell layer covering dermal papillae, while podoplanin (red) is marked at the basal cell layer of rete ridges in psoriatic hyperproliferative epidermis (*P* dermal papillae, *R* rete ridges). The broken lines indicate dermo-epidermal junction. **b** Podoplanin (red) is marked at the basal cell layer of rete ridges, while ECM-bound active β1-integrin (green) is marked at the basal cell layer covering the dermal papillae in psoriatic epidermis (*P* dermal papillae, *R* rete ridges). The broken lines indicate dermo-epidermal junction

suppression of ECM-bound β1-integrin is still elusive, CD44 can be a communal factor between podoplanin and β1-integrin. CD44, a ubiquitous hyaluronan receptor, is a partner molecule of both podoplanin and β1-integrin. Interaction of CD44 with the extracellular domain of podoplanin results in directed cell migration and interaction with β1-integrin induces cell survival through Src kinase activation [19]. Because the expression of total CD44 is not altered in the psoriatic hyperproliferative epidermis [28], the functional alteration of β1-integrin may depend on the competitive inhibition of the interaction between β1-integrin and a CD44 variant by podoplanin. These findings observed in the normal epidermal keratinocytes are inconsistent with those in other types of cells, such as oral squamous cell carcinoma cells [30] and lymphatic endothelial cells [5]. While the detailed molecular mechanism relating to the difference has not been fully elucidated, the podoplanin-mediated differentiating mechanism in hyperproliferative keratinocytes might contribute to maintain a polarized architecture of the normal epidermis.

Podoplanin is upregulated at the basal cell layer of the psoriatic hyperproliferative epidermis displaying a decreased turnover time. In this condition, both cell proliferation and differentiation must be rebalanced in an accelerated manner. The podoplanin-mediated inhibition of β1-integrin functions in association with keratinocyte differentiation may be significantly involved in this process. For example, the podoplanin-positive and loosely bound ECM cells would be more easily detached from the basal cell layer and would differentiate under horizontally compressive conditions of the psoriatic hyperproliferative epidermis. Further studies are required to verify the podoplanin-functions in keratinocytes; however, this novel podoplanin-dependent downregulation mechanism of β1-integrin may be significantly involved in the pathophysiology of psoriasis, where both accelerated detachment and differentiation of keratinocytes are required to maintain the characteristic epidermal remodeling process.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was conducted according to the Declaration of Helsinki and the study protocol was approved by the Asahikawa Medical University ethical committee.

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