

Expression of the proteoglycan syndecan-4 and the mechanism by which it mediates stress fiber formation in folliculostellate cells in the rat anterior pituitary gland

Kotaro Horiguchi, Tom Kouki, Ken Fujiwara, Takehiro Tsukada, Floren Ly, Motoshi Kikuchi and Takashi Yashiro

Division of Histology and Cell Biology, Department of Anatomy, Jichi Medical University School of Medicine, 3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, Japan

(Correspondence should be addressed to T Yashiro; Email: tyashiro@jichi.ac.jp; K Horiguchi; Email: kota@jichi.ac.jp)

Abstract

Folliculostellate (FS) cells in the anterior pituitary gland appear to have multifunctional properties. FS cells connect to each other at gap junctions and thereby form a histological and functional network. We have performed a series of studies on network formation in FS cells and recently reported that FS cells markedly prolong their cytoplasmic processes and form numerous interconnections with neighboring FS cells in the presence of laminin, an extracellular matrix (ECM) component of the basement membrane. In this study, we investigated the mechanism of this extension of FS cell cytoplasmic processes under the influence of laminin and found that laminin promoted stress fiber formation within FS cells. Next, we noted that formation of stress fibers in FS cells

was mediated by syndecan-4, a transmembrane proteoglycan that binds ECM and soluble factors via their extracellular glycosaminoglycan chain. We then observed that expressions of syndecan-4 and α -actinin (a microfilament bundling protein that cross-links actin stress fibers in FS cells) were upregulated by laminin. Using specific siRNA of syndecan-4, actin polymerization of FS cells was inhibited. Our findings suggest that FS cells received a signal from laminin–syndecan-4 interaction, which resulted in morphological changes, and that the formation of a morphological and functional network in FS cells was transduced by a syndecan-4-dependent mechanism in the presence of ECM.

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Introduction

The anterior pituitary gland comprises five types of hormone-producing cells plus folliculostellate (FS) cells, which do not produce classical anterior pituitary hormones. Histologically, these six types of anterior pituitary cells aggregate and form clusters surrounded by different types of extracellular matrix (ECM), which provide the necessary mechanical integrity, rigidity, and elasticity for these cells to perform their respective roles (Soji & Herbert 1989, Kaidzu *et al.* 2000, Paez-Pereda *et al.* 2005). It has been shown that lobular structures surrounded by ECM are responsible for organizing aggregations of anterior pituitary cells into a functional unit in the anterior pituitary gland (Soji & Herbert 1989, Shirasawa *et al.* 2004). FS cells are located in the core of these lobular structures.

Accumulating evidence indicates that FS cells have numerous possible functions. They have been reported to act as stem cells, phagocytes, and cells that regulate hormone release (Inoue *et al.* 1999, Allaerts & Vankelecom 2005). Regarding the histological features of FS cells in the anterior pituitary gland, FS cells are interconnected via their

cytoplasmic processes and formed gap junctions at their adhesion sites (Morand *et al.* 1996, Sato *et al.* 2005). The network formed by these connections is referred to morphologically as the FS cell network (Sato *et al.* 2005). In addition, by means of Ca^{2+} waves through the gap junction channels, FS cells stimulate the building of a large-scale communication network with the neighboring FS cells in the anterior pituitary gland (Fauquier *et al.* 2001, Sato *et al.* 2005). These findings have led to speculation regarding the function of FS cells. One hypothesis is that FS cells transmit signals via gap junctions so as to regulate hormone release from hormone-producing cells, in addition to the established hypophyseal–portal system (Shirasawa *et al.* 2004, Sato *et al.* 2005).

We conducted a series of studies that used living-cell imaging in primary culture of anterior pituitary cells to investigate FS cell network formation and found that FS cells extend and contract their cytoplasmic processes by rearranging their actin cytoskeleton, thereby establishing interconnections between FS cells (Horiguchi *et al.* 2010). Furthermore, we showed that FS cells in the presence of ECM markedly prolong their cytoplasmic processes, form numerous

interconnections with neighboring FS cells, and promote assembly of the FS cell network (Horiguchi *et al.* 2010, 2011a). However, it is not clear how FS cells under the influence of ECM prolong their cytoplasmic processes and initiate network construction with other FS cells in primary culture. By means of signal transduction, ECM arranges the actin cytoskeleton via families of transmembrane receptors called integrins (Hynes 2002) and syndecans (Parsons *et al.* 2000, Dovas *et al.* 2006, Xian *et al.* 2010). In this study, we therefore attempted to identify the mechanism responsible for the extension of FS cell cytoplasmic processes. First, we observed stress fiber formation in FS cells in the presence of laminin, an ECM component of the basement membrane. Stress fibers are composed of bundles of 10–30 actin filaments, and these bundles are held together with the actin-crosslinking protein α -actinin (Pellegrin & Mellor 2007). On the basis of their subcellular location, observed stress fibers are grouped into three classes, i.e. ventral stress fibers, dorsal stress fibers, and transverse arcs (Small *et al.* 1998). Ventral stress fibers are the most common and are located along the base of the cytoplasm (Pellegrin & Mellor 2007). Finally, we analyzed the downstream events of ECM signaling with mediation by syndecan-4, which is a single-pass transmembrane proteoglycan that binds a number of signaling molecules potentially linked with stress fiber formation.

Materials and Methods

Animals

Transgenic S100b-GFP rats that express green fluorescent protein (GFP) under the control of the promoter of S100 β protein gene – a marker of FS cells – were donated by Prof. K Inoue of Saitama University and bred in our laboratory (Itakura *et al.* 2007). Eight- to ten-week-old male rats weighing 250–300 g were given *ad libitum* access to food and water and housed under conditions of a 12 h light:12 h darkness cycle. Rats were killed by exsanguination from the right atrium under deep pentobarbital anesthesia and then perfused with Ca^{2+} - and Mg^{2+} -free Hanks' solution for primary culture. All animal experiments were performed after receiving approval from the Institutional Animal Experiment Committee of Jichi Medical University and were conducted in accordance with the Institutional Regulation of Animal Experiments and Fundamental Guideline for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions, under the jurisdiction of the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

Cell culture

Anterior pituitary cells of male S100b-GFP rats were dispersed as described previously (Horiguchi *et al.* 2008). Dispersed cells were then plated onto eight-well glass chamber slides (1 cm²/well; Nalge Nunc Int., Rochester, NY, USA) with or without a

coating of laminin (10 $\mu\text{g}/\text{cm}^2$; Millipore, Bedford, MA, USA) at a density of 1×10^5 cells/cm² in 400 μl of Medium 199 with Earle's salts (Invitrogen) supplemented with 10% fetal bovine serum (Sigma–Aldrich Corp.), 0.5 U/ml of penicillin, and 0.5 $\mu\text{g}/\text{ml}$ of streptomycin (Invitrogen). Other dispersed cells were separated into GFP-positive and GFP-negative cells by a cell sorter (MoFlo XDP; Beckman Coulter, Inc., Fullerton, CA, USA). GFP-positive cells were plated onto eight-well glass chamber slides (Nalge Nunc Int.), with or without a coating of 10 $\mu\text{g}/\text{cm}^2$ of laminin at a density of 1×10^5 cells/cm². Cells were then cultured for 72 h at 37 °C in a humidified atmosphere of 5% CO₂ and 95% air.

Fluorescent cytochemistry of actin filaments

To observe cellular localization of actin filaments, cells were fixed with 4% paraformaldehyde in 25 mM phosphate buffer (PB, pH 7.4) for 20 min and permeabilized with 0.5% Triton X-100 in PBS for 5 min at room temperature. Fixed cells were incubated with 0.1 μM rhodamine-phalloidin (Cytoskeleton, Inc., Denver, CO, USA) for 30 min at room temperature and washed with PBS. Cells were scanned at a thickness of 1 μm using a confocal laser microscope (FV1000; Olympus Corp., Tokyo, Japan).

Electron microscopic observation

For observation of stress fiber, FS cells in primary culture on laminin-coated surfaces were fixed with 2.5% glutaraldehyde in 0.1 M PB, pH 7.4, for 1 h at 4 °C. Then, FS cells were postfixed with 1% OsO₄ in 0.1 M PB for 1 h at 4 °C, dehydrated in a series of graded alcohols, and embedded in epoxy resin (Quetol 812; Nissin EM Co., Tokyo, Japan). FS cells were sectioned into ultrathin slices using a Reichert-Nissei Ultracut S (Leica Microsystems, Wetzlar, Germany), stained with uranyl acetate and lead citrate, and then observed under a Hitachi H-7600 electron microscope (Hitachi).

Immunoblot analysis

After primary culture, cells on six wells were washed in PBS and lysed in RIPA buffer (20 mM Tris, 150 mM NaCl, 2 mM EDTA, 0.1% v/v SDS, 1% v/v Triton X-100, pH 7.5), and total protein was estimated by using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. Twenty micrograms of protein from each sample were applied to 12% SDS-PAGE. Proteins were then transferred electrophoretically to Immobilon-P transfer membrane (Millipore). The membrane was blocked with 5% (w/v) nonfat dried milk in TBST (50 mM Tris, 100 mM NaCl, 0.1% v/v Tween 20, pH 7.4) for 1 h. After washing with TBST, the membrane was incubated overnight with mouse monoclonal α -actinin (1:5000; Enzo Life Sciences, Inc., Farmingdale, NY, USA) or rabbit polyclonal syndecan-4 (1:10 000; Assay Biotechnology Company, Inc., Sunnyvale, CA, USA) antibodies, diluted in Can Get Signal

Solution (Toyobo, Osaka, Japan), followed by TBST washes, and incubated for 1 h with HRP-labeled secondary antibodies (Envision+System-HRP, anti-rabbit, Dako, Glostrup, Denmark). After washing with TBST, specific immunoreactivity was visualized using a Chemiluminescence ECL Plus System (GE Healthcare, Mississauga, ON, Canada) with lumi-shot film (Fujifilm, Tokyo, Japan). The film was scanned, and densitometric analysis was performed with ImageJ Software (National Institutes of Health, Bethesda, MD, USA). The results were normalized with β -actin. Each analysis was performed in triplicate.

Quantification of mRNA levels by real-time RT-PCR

Total RNA fractions were prepared, and cDNA was synthesized as described previously (Horiguchi *et al.* 2011a). Quantitative real-time PCR (ABI PRISM 7900HT; Applied Biosystems) was performed by using gene-specific primers and SYBR Premix Ex Taq (Takara, Tokyo, Japan) containing SYBR Green I. The following primers were used to amplify cDNA fragments of syndecan-4 (GenBank accession no. NM_012649): forward 5'-GCCTTGAGCAGGACTCTGAC-3' and reverse 5'-TTCTCGGGGATGTGGTTATC-3' (124 bp); α -actinin (BC074001): forward 5'-CAACATGGAGAGGCAGAAT-3' and reverse 5'-TCCGCTGTATCTGTGTCAGC-3' (126 bp); PKCa (NM_001105713): forward 5'-ATGGCACACATGGAGCTGTA-3' and reverse 5'-ATGCAAAATCCATTGCATGA-3' (118 bp); FAK (ENSRNOT00000011219): forward 5'-CCTGTGGATCCTGAAGCATT-3' and reverse 5'-TCTTGATGCAGTTGGAGTG-3' (116 bp). For normalization, we also quantified glyceraldehyde 3-phosphate dehydrogenase (GAPDH, M_17701): forward 5'-AAGGGCTCATGACCACAGTC-3'

and reverse 5'-GGATGCAGGGATGATGTTCT-3' (116 bp). Relative quantification was conducted using the standard curve method and was performed in triplicate.

siRNA for syndecan-4

For siRNA transfection, the culture medium was replaced by 400 μ l Medium 199 with Earle's salts (Invitrogen) supplemented with 10% fetal bovine serum (Sigma-Aldrich Corp.), 0.5 U/ml penicillin, and 0.5 μ g/ml streptomycin (Invitrogen), siRNA against syndecan-4 mRNA (0.2 μ M, Rn_Sdc4_7; Qiagen), and INTERFERin (1:100 v/v) transfection reagent at 24 h. Non-silencing siRNA with no homology to any known mammalian gene was used as a negative control (Qiagen). After that, the GFP-positive cells were cultured for 48 h, and 2×10^5 and 6×10^5 cells were used for real-time RT-PCR and immunoblot analysis respectively. For observation of actin filaments, we retrieved the GFP-positive cells at 48 h using Ca^{2+} - and Mg^{2+} -free Hanks' solution containing 0.3% ethylenediaminetetraacetic acid (Wako Pure Chemicals, Osaka, Japan) for 20 min at 37 °C, and redispersed GFP-positive cells were cultured on laminin-coated surface for 24 h. After that, the GFP-positive cells were fixed for the above-mentioned rhodamine-phalloidin staining. Each analysis was performed in triplicate.

Immunocytochemistry

Cultured cells fixed with 4% paraformaldehyde in 50 mM PB for 20 min at room temperature were first immersed in PBS containing 2% normal goat serum for 20 min at 30 °C, then incubated with α -actinin (1:200; Enzo Life Sciences, Inc.) or rabbit polyclonal syndecan-4 (1:400; Assay Biotechnology Company, Inc.) antibodies for overnight at room

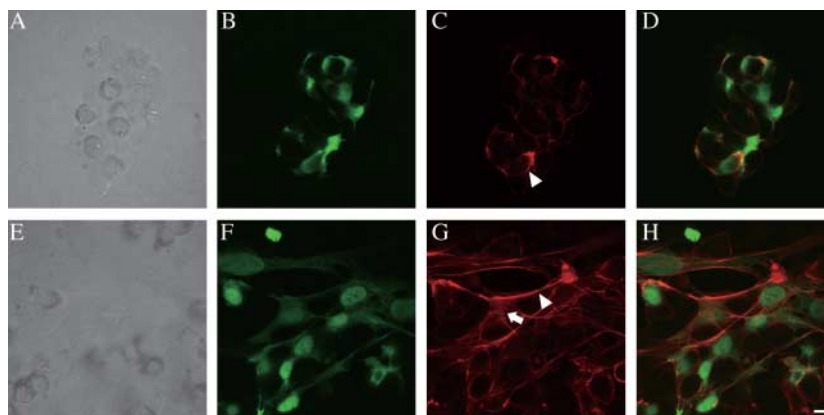


Figure 1 Stress fibers in primary culture of anterior pituitary cells on uncoated and laminin-coated surfaces. (A, B, C and D) Primary culture on uncoated surface at 72 h. (E, F, G and H) Primary culture on laminin-coated surface at 72 h. (A and E) Phase-contrast images. (B and F) GFP images (green, FS cells). (C and G) Rhodamine-phalloidin staining (red, stress fiber). (D and H) Superimposition of B and C and F and G respectively. Ventral or dorsal stress fibers (arrow) formed in the cytoplasm of FS cells, and transverse arcs formed beneath the cell membrane (arrowhead). Scale bar, 10 μ m.

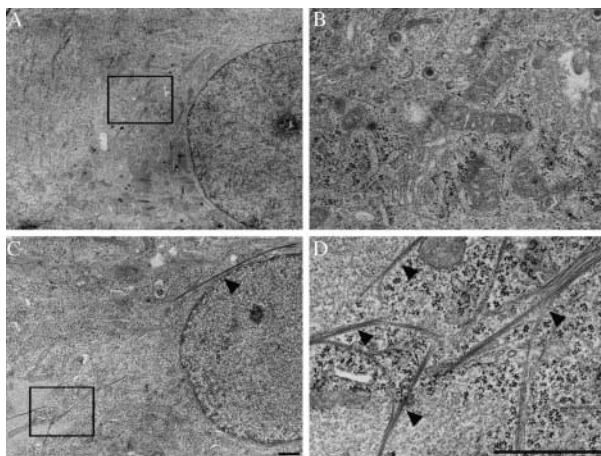


Figure 2 Electron micrographs of FS cells cultured on uncoated and laminin-coated surfaces for 72 h. (A) Lower magnification on uncoated surface. (B) Higher magnification of area indicated by the box in A. (C) Lower magnification on laminin-coated surface. (D) Higher magnification of area indicated by the box in C. Bundles of parallel microfilaments were present (arrowheads). Scale bars, 500 nm.

temperature. After washing with PBS, cells were incubated in PBS with Alexa Fluor 568-conjugated goat anti-mouse IgG (Invitrogen) diluted to 1:200. Absence of an observable nonspecific reaction was confirmed using normal mouse or rabbit serum. Cells were scanned at a thickness of 1 μ m using a confocal laser microscope (FV1000; Olympus Corp.).

Statistical analysis

Results are presented as mean \pm S.E.M. The *t*-test was used to compare differences between two groups. A *P* value < 0.05 was considered to indicate statistical significance.

Results

Laminin-induced stress fiber formation in FS cells

We used rhodamine-phalloidin staining to observe the filamentous actin arrangement of anterior pituitary cells in primary culture. On the uncoated surface, actin filaments were detected along the elongated cytoplasmic processes of FS cells (Fig. 1A, B, C and D, arrowhead). However, actin filaments were far more numerous in the cytoplasm and cytoplasmic processes of FS cells on the laminin-coated surface (Fig. 1E, F, G and H, arrow and arrowhead), which suggest that stress fibers formed in the cytoplasm of FS cells on the laminin-coated surface. GFP-negative cells, which are primarily hormone-producing cells, retained their round shape and did not form stress fibers on either the uncoated or laminin-coated surfaces (Fig. 1).

Electron microscopic observation of FS cells

To verify the presence of actin stress fibers in FS cells, we used an electron microscope to observe FS cells in primary culture

on uncoated (Fig. 2A and B) and laminin-coated surfaces (Fig. 2C and D). There were few actin filaments in the cytoplasm of FS cells on the uncoated surface (Fig. 2B). By contrast, we noted bundles of parallel microfilaments in FS cells on the laminin-coated surface (Fig. 2C and D).

Expression of syndecan-4 in FS cells

A cell sorter was used to isolate FS cells from male S100b-GFP rat anterior pituitary for real-time RT-PCR and western blotting. To determine whether FS cells express syndecan-4, we performed quantitative real-time RT-PCR. Syndecan-4 expression was significantly higher in GFP-positive cells than in GFP-negative cells (Fig. 3A). In western blot analysis, syndecan-4 immunoreactivity was also detected in FS cells as an \sim 22 kDa band, which conforms to the expected size of rat syndecan-4 core protein (Fig. 3B).

Change in syndecan-4 mRNA and protein expression in FS cells on laminin

To determine whether laminin affects syndecan-4 and α -actinin gene expression in FS cells, we performed quantitative real-time RT-PCR. α -Actinin is a 100 kDa microfilament bundling protein that cross-links actin stress fibers in muscle and nonmuscle cells (Lazarides & Burridge 1975, Otey & Carpen 2004). As compared with the uncoated surface, gene expressions of syndecan-4 and α -actinin were higher in cells on the laminin-coated surface at 72 h (Fig. 4A and B). However, the expressions of protein kinase C α (PKC α) and focal adhesion kinase (FAK), which are signaling molecules of syndecan-4 and integrin β 1 in stress fiber assembly, were not higher in cells on the laminin-coated surface (Fig. 4C and D). We also examined localization of

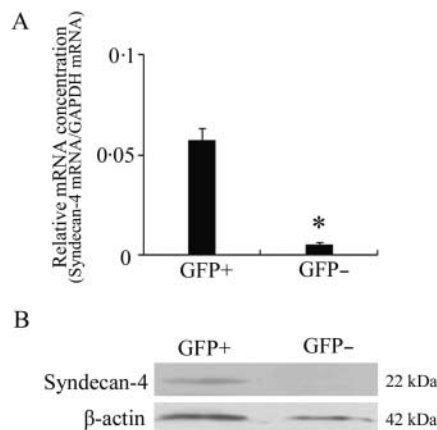


Figure 3 Syndecan-4 expression in FS cells. (A) Expression of syndecan-4 mRNA in GFP-positive (GFP+, FS cells) and GFP-negative (GFP-) cells, as determined by real-time RT-PCR, was normalized with an internal control (GAPDH; mean \pm S.E.M., *n*=3). **P*<0.01. (B) Syndecan-4 protein was analyzed by western blotting. The upper panel shows syndecan-4 and the lower panel shows β -actin (loading control).

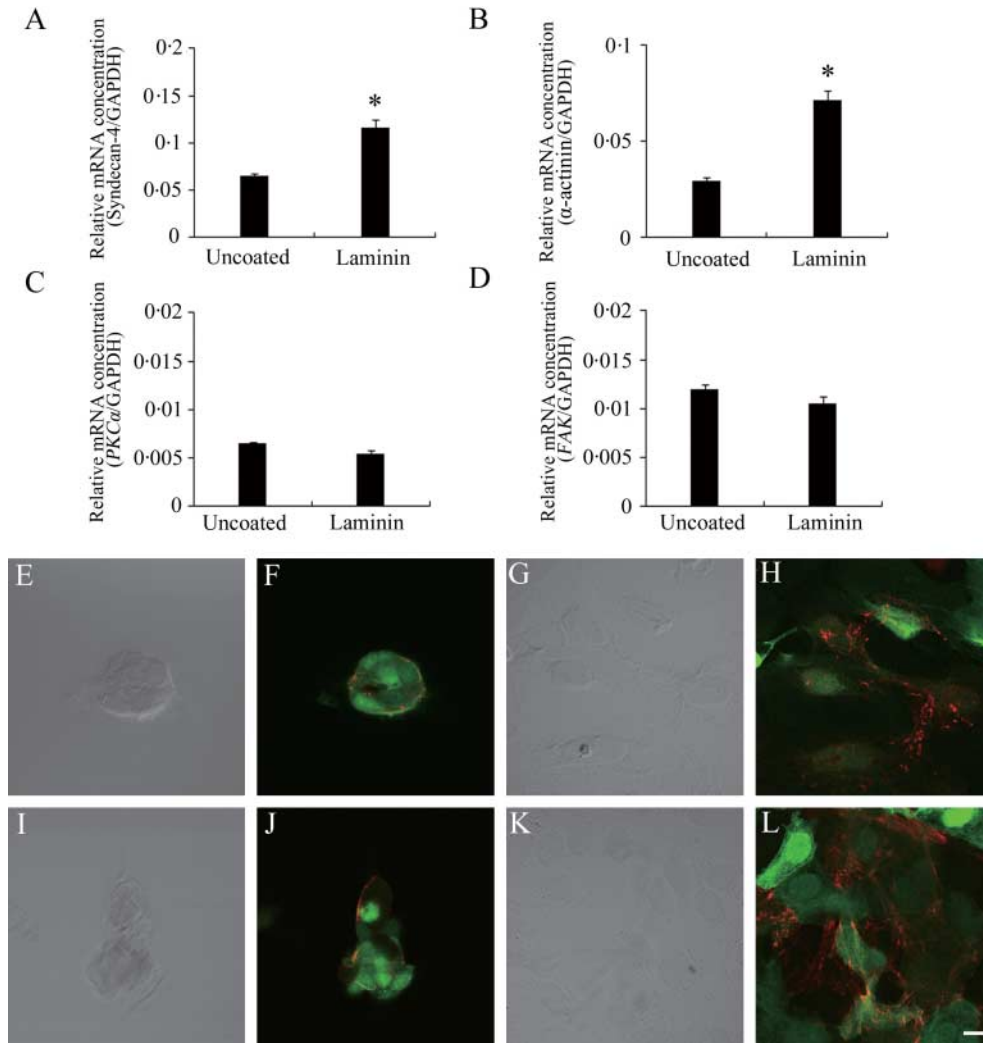


Figure 4 Syndecan-4 expression in primary culture of FS cells after 72-h incubation. (A, B, C and D) Expressions of syndecan-4, α -actinin, *PKC α* , and *FAK* mRNA, respectively, as determined by real-time RT-PCR, were normalized with an internal control (GAPDH; mean \pm s.e.m., $n=4$). Syndecan-4 and α -actinin mRNA expression was significantly higher on the laminin-coated surface (laminin) than on the uncoated surface (uncoated). * $P<0.01$. (E, F, G and H, and I, J, K and L) Immunocytochemistry of syndecan-4 and α -actinin in primary culture of FS cells respectively. (E, F, I and J) 72-h culture of FS cells on uncoated surface. (G, H, K and L) 72-h culture of FS cells on laminin-coated surface. (E, G, I and K) Phase-contrast images. (F, H, J and L) Fluorescent images. Syndecan-4 and α -actinin (red) immunoreactivity was observed in FS cells (green). Scale bar, 10 μ m.

syndecan-4 and α -actinin by immunocytochemistry at 72 h in primary culture on uncoated and laminin-coated surfaces. Immunoreactive syndecan-4 and α -actinin were located on cytoplasmic processes of FS cells on both the uncoated and laminin-coated surfaces (Fig. 4E, F, G, H, I, J, K, and L). Syndecan-4 and α -actinin immunoreactivity in FS cells was clearly greater on the laminin-coated surface (Fig. 4H and L) than on the uncoated surface (Fig. 4F and J).

Knockdown of syndecan-4 by siRNA

To examine the functional role of syndecan-4 in stress fiber formation in FS cells, we used siRNA to downregulate

syndecan-4 expression in FS cells on the laminin-coated surface. When syndecan-4 was downregulated with a specific siRNA, its expression decreased (Fig. 5A, C and D). In addition, quantitative analysis revealed that α -actinin expression in syndecan-4 siRNA-treated FS cells was lower than in control FS cells (Fig. 5B, C and E). Finally, rhodamine-phalloidin staining was used to examine the effect of syndecan-4 silencing on stress fiber formation in FS cells and, in contrast with control (Fig. 5F, G, H and I), ventral and dorsal stress fibers were not observed in FS cells after syndecan-4 downregulation (Fig. 5J, K, L and M). However, we did observe actin filaments beneath cell membranes (transverse arcs) of FS cells after syndecan-4 silencing (Fig. 5J, K, L and M).

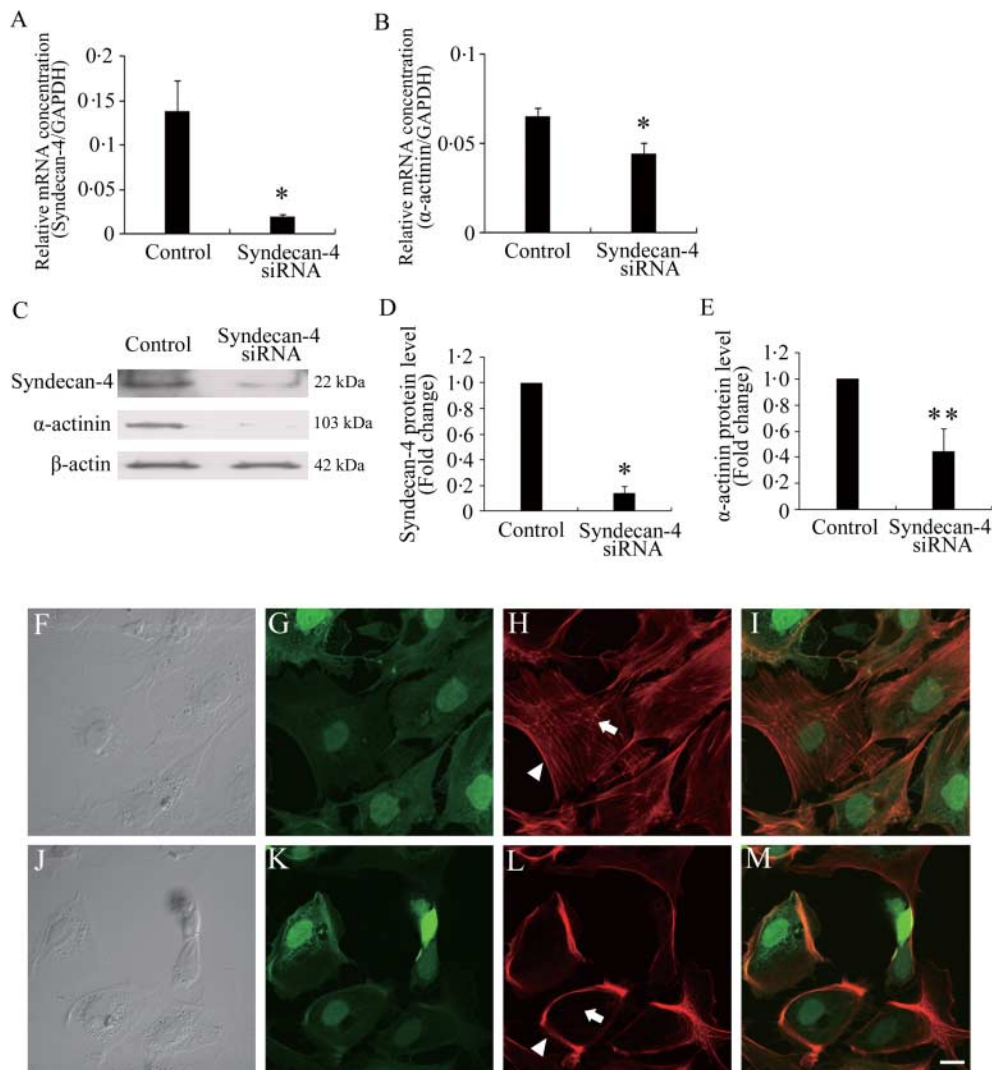


Figure 5 Downregulation of syndecan-4 by siRNA. (A and B) Expressions of syndecan-4 and α -actinin mRNA, respectively, as determined by real-time RT-PCR, were normalized with an internal control (GAPDH) for 48-h incubation after FS cells were transfected with siRNA (mean \pm S.E.M., $n=4$). Syndecan-4 and α -actinin mRNA expression was significantly lower in the experimental group than in the control. (C) The protein levels of syndecan-4 and α -actinin were determined by western blotting. The top panel shows syndecan-4, the middle panel shows α -actinin, and the bottom panel shows β -actin as the loading control. (D and E) Western blotting data from three experiments (mean \pm S.E.M., $n=3$) were quantified by densitometry and normalized with β -actin. Levels of syndecan-4 and α -actinin protein (syndecan-4 siRNA) were calculated as ratios of control values (Control). After siRNA treatment, the protein level of syndecan-4 (D) and α -actinin (E) in FS cells (syndecan-4 siRNA) was significantly lower than in control (Control). * $P<0.01$; ** $P<0.05$. (F, G, H and I) Rhodamine-phalloidin staining for 48-h incubation after FS cells were transfected by siRNA (F, G, H and I, control siRNA and J, H, I, J, K, L and M, syndecan-4 siRNA). (F and J) Phase-contrast images. (G and K) GFP images (green, FS cells). (H and L) Rhodamine-phalloidin staining (red, stress fiber). (I and M) Superimposition of G and H, and K and L respectively. Ventral and dorsal stress fiber formation (arrow) in the cytoplasm of FS cells was inhibited by siRNA specific for syndecan-4, but formation of transverse arcs beneath the cell membrane of FS cells (arrowhead) was not inhibited. Scale bar, 10 μ m.

Discussion

In this study, we observed that morphological changes in FS cells in the presence of laminin were strongly dependent on increased stress fiber formation. We also found that stress fiber formation was transduced by a syndecan-4-mediated mechanism in FS cells.

In a recent study, we showed that laminin markedly increased the extension of cytoplasmic processes and their interconnections in FS cells (Horiguchi *et al.* 2010). In this study, we found that actin filaments were more numerous in FS cells in the presence of laminin (Fig. 1). Furthermore, we isolated pure FS cells from the anterior pituitary of S100b-GFP rat, so as to eliminate the physical and functional effects

of hormone-producing cells. This allowed us to examine the direct involvement of laminin on actin polymerization of FS cells. As shown in Figs 2 and 4, it is clear that laminin directly affected the extension of cytoplasmic processes by means of stress fiber formation in FS cells.

ECM transduces signals to cells through the integrin family of cell-surface receptors (Hynes 2002). Cell-ECM adhesion through integrin is the starting point for stress fiber formation in cells (Defilippi *et al.* 1999, Wiesner *et al.* 2005). Integrins comprise the α - and β -subunits, which can form heterodimers. In mammals, 18 α -integrin and eight β -integrin subunits have been described, and these can associate in a heterodimeric fashion to form at least 24 different receptors (Hynes 2002). We reported that FS cells bind laminin through integrin- $\alpha 3\beta 1$ and/or - $\alpha 6\beta 1$ and that an antibody that inhibits the integrin- $\beta 1$ cascade blocked the extension of cytoplasmic processes in FS cells (Horiguchi *et al.* 2011a,b). There is little doubt that stress fiber formation in FS cells also depends on FS cell-laminin adhesion through integrin- $\alpha 3\beta 1$ and/or - $\alpha 6\beta 1$. However, there are several signal transduction pathways after cells adhere to ECM through integrin- $\beta 1$, including FAK (Parsons *et al.* 2000) and syndecan-4-mediated PKC α (PRKCA) signaling (Dovas *et al.* 2006, Pellegrin & Mellor 2007), which lead to α -actinin transcription and contribute to stress fiber formation. Syndecans are a family of transmembrane proteoglycans that bind ECMs and soluble factors (Simons & Horowitz 2001). A number of studies have shown that syndecan-4, which acts as a cell-surface receptor via its extracellular glycosaminoglycan chains, is involved in actin polymerization (Simons & Horowitz 2001). Syndecan-4 is expressed in fibroblasts (Sawaguchi *et al.* 2006), skeletal and smooth muscle cells (Cornelison *et al.* 2001, Li & Chaikof 2002), chondrocytes (Barre *et al.* 2000), and Sertoli cells (Brucato *et al.* 2000). As shown in Figs 3 and 4, FS cells in the anterior pituitary also expressed syndecan-4, and its expression increased in the presence of laminin. Furthermore, although ventral and dorsal stress fiber formation in FS cells was inhibited by siRNA specific for syndecan-4, transverse arc formation was not inhibited (Fig. 5). These results suggest that actin polymerization or arrangement in FS cells was mediated by syndecan-4 after cells adhered to ECM through integrin. However, the mechanism responsible for laminin-induced syndecan-4 upregulation in FS cells remains to be elucidated.

It is interesting that the expressions of FAK and PKC α were not upregulated by laminin in FS cells (Fig. 4). Greene *et al.* (2003) reported that syndecan-4 is able to influence the actin cytoskeleton because α -actinin directly binds the cytoplasmic domain. Because of its unusual molecular structure, α -actinin contributes to stability and plasticity in a variety of actin-based arrangements. It forms an antiparallel dimer with an actin-binding site at the N-terminus of each monomer (Otey & Carpen 2004). We therefore speculate that FAK and PKC α signaling do not have roles in stress fiber formation in FS cells. Recently, we reported that hormone-producing cells also

expressed integrin- $\beta 1$ and observed little morphological change or extension of cytoplasmic processes of these cells in the presence of laminin (Horiguchi *et al.* 2010). Thus, the difference in the response to laminin is likely due to syndecan-4 expression (Fig. 3). Our present findings indicate that the syndecan-4-mediated mechanism was specific to FS cells in the anterior pituitary gland.

In the anterior pituitary gland, the lobular structures surrounded by ECM have a role in cell aggregation (Soji & Herbert 1989, Shirasawa *et al.* 2004). Knowledge of the topographic features of FS cells is essential in understanding these lobular structures, the location of FS cells in the core of the lobular structure, and the characteristic envelopment of hormone-producing cells by their cytoplasmic processes, the tips of which attach to the ECM (Soji & Herbert 1989, Inoue *et al.* 1999, Shirasawa *et al.* 2004). In our studies on FS cell activity in the presence of ECM (Horiguchi *et al.* 2010, 2011a,b), we have shown that FS cells markedly extend their cytoplasmic processes and form networks at gap junctions. In this study, we have shown that FS cells under the influence of laminin extend their cytoplasmic processes due to a syndecan-4-dependent mechanism that leads to activation of α -actinin in FS cells. These findings suggest that FS cells have important functional roles in determining and/or maintaining local cellular arrangement in the presence of ECM. Future study is needed to investigate the action of hormone-producing cells and their behavior in the presence of ECM.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

K H collected data, contributed to the Discussion, wrote the manuscript, and reviewed and edited the manuscript. T K collected data. K F collected data and contributed to the Discussion. T T collected data and contributed to the Discussion. L F reviewed and edited the manuscript. M K reviewed and edited the manuscript. T Y contributed to the Discussion, wrote the manuscript, and reviewed and edited the manuscript.

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