Japan Society of Histochemistry and Cytochemistry
Established 1959

Acta Histochem. Cytochem. 51 (5): 145–152, 2018
doi: 10.1267/ahc.18014

Immunohistochemical Study of the Laminin α5 Chain and Its Specific Receptor, Basal Cell Adhesion Molecule (BCAM), in both Fetal and Adult Rat Pituitary Glands

Morio Azuma[1], Takehiro Tsukada[2], Takeshi Inagaki[3], Fujianti Casmad[1], Depicha Jindatip[1,4], Alimuddin Tofrizal[1], Rita Maliza[1], Khongorzul Batchuluun[1], Rahimi Syaidah[1], Nobuhiko Ohno[1], Ken Fujiwara[1], Motoshi Kikuchi[1,5] and Takashi Yashiro[1]

[1]Division of Histology and Cell Biology, Department of Anatomy, Jichi Medical University School of Medicine, Tochigi, Japan, [2]Department of Biomolecular Science, Faculty of Science, Toho University, Chiba, Japan, [3]Division of Forensic Medicine, Department of Anatomy, Jichi Medical University School of Medicine, Tochigi, Japan, [4]Department of Anatomy, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand and [5]Laboratory of Natural History, Jichi Medical University School of Medicine, Tochigi, Japan

Received March 16, 2018; accepted August 10, 2018; published online September 13, 2018

Laminin, a major basement membrane protein, comprises three subunit chains: α, β, and γ chains. Among these chains, only the laminin α chain is capable of signaling via laminin receptors. Although laminin isoforms containing the α5 chain were reported to be the first laminin produced during rat anterior pituitary gland development, the functions of these isoforms are unknown. We used immunohistochemical techniques to localize the laminin α5 chain and its specific receptor, basal cell adhesion molecule (BCAM), in fetal and adult pituitary gland. Laminin α5 chain immunoreactivity was observed in the basement membrane of the primordial adenohypophysis at embryonic days 12.5 to 19.5. Double immunostaining showed that BCAM was present and co-localized with the laminin α5 chain in the tissue. Quantitative analysis showed that the laminin α5 chain and BCAM were expressed in the anterior pituitary gland during postnatal development and in adulthood (postnatal day 60). In the adult gland, co-localization of the laminin α5 chain and BCAM was observed, and BCAM was detected in both the folliculo-stellate cells and endothelial cells. These results suggest that laminin α5 chain signaling via BCAM occurs in both the fetal adenohypophysis and adult anterior pituitary gland.

Key words: laminin, basal cell adhesion molecule, laminin receptor, pituitary development, immunohistochemistry

I. Introduction

The basement membrane is an extracellular scaffold for cells, aids in structural support of many tissues, and also functions as a barrier between tissues. Cell-basement membrane interactions are involved in proliferation, adhesion, migration, and differentiation of cells during embryonic morphogenesis and in adult tissues. The basement membrane has four major components: laminin, type IV collagen, nidogen, and heparan sulfate proteoglycan [26]. Laminin is required for basement membrane assembly and is made up of three subunits, namely, α, β, and γ chains [1, 4]. There are five α (α1-5), three β (β1-3), and three γ (γ1-3) chains, and combinations of these chains assemble into 19 different laminin isoforms [6]. Among these laminin chains, the α chains are thought to be the major deter-

Correspondence to: Takashi Yashiro, M.D., Ph.D. and Morio Azuma, Ph.D., Department of Anatomy, Jichi Medical University School of Medicine, 3311–1 Yakushiji, Shimotsuke, Tochigi 329–0498, Japan.
E-mail: tyashiro@jichi.ac.jp (Takashi Yashiro) and azumam@jichi.ac.jp (Morio Azuma)

minant of laminin function, since they have C-terminal laminin globular (LG) domains, which bind the laminin receptors [26].

The anterior pituitary gland is composed of five types of hormone-secreting cells and folliculo-stellate cells, which do not produce classical anterior pituitary hormones. The gland arises from a primordial tissue termed Rathke's pouch, a thickened primordial oral ectoderm in the embryo. Recently, using *in situ* hybridization, our research group showed that laminin chain expression differed in the embryonic and postnatal rat pituitary gland [21]. In that study, laminin isoforms containing the α5 chain appeared to have an important role in gland development, since the laminin α5 chain is the first α chain expressed by primordial tissue in the gland. The function of laminin isoforms containing the α5 chain is believed to involve enhancing the structural integrity of the basement membrane during organogenesis [23]. In addition, recombinant laminins containing the α5 chain were involved in self-renewal of embryonic stem cells [5]. However, little is known of the distribution of the laminin α5 chain or the existence of its receptor in the pituitary gland. Furthermore, the function of laminin isoforms containing the α5 chain remains unclear. Using immunohistochemical techniques, we investigated the distribution of the laminin α5 chain and its specific receptor, basal cell adhesion molecule (BCAM), in both fetal and adult rat pituitary glands.

II. Materials and Methods

Animals

Adult Wistar rats were purchased from Japan SLC (Shizuoka, Japan). The animals were maintained under a 12-hr light/dark cycle and given conventional food and water *ad libitum*. Room temperature was maintained at approximately 22°C. The day on which spermatozoa were identified in a vaginal smear was designated as embryonic day 0.5 (E0.5). The date of birth was designated as postnatal day 0 (P0). 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 Regulations for Animal Experiments and Fundamental Guidelines 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.

Tissue preparation

Cryosections of freshly frozen tissue were used for immunostaining the laminin α5 chain. Pregnant rats were anesthetized with pentobarbital sodium (25 mg per kg body weight, i.p., Kyoritsu Seiyaku, Tokyo, Japan) when their fetuses reached E12.5, E13.5, E14.5, E15.5, E16.5, E17.5, E18.5, and E19.5. Pituitary glands were excised from P3 and P60 male rats under deep anesthesia. At each stage, four animals were used for analysis. The resected fetuses and excised pituitary glands were embedded in Tissue-Tek OTC compound and frozen in liquid nitrogen. Sagittal sections of fetuses and frontal sections of the pituitary glands (thickness 4 μm) were obtained with a cryostat (CM3000; Leica Microsystems, Wetzlar, Germany) and mounted on glass slides. Before immunohistochemistry, sections were fixed in ice-cold methanol for 10 min.

The pituitary glands were excised from four P60 male rats that were perfused through the left ventricle with 4% paraformaldehyde in 0.05 M phosphate buffer (pH 7.4) for 5 min under anesthesia. The glands were then immersed in the same fixative for 24 hr at 4°C. The glands were subsequently dehydrated, embedded in paraffin, and cut into frontal sections (thickness 2 μm) with an ultramicrotome (Ultracut UCT; Leica Microsystems). After deparaffinization, sections were incubated in an Immunosaver (Nisshin EM, Tokyo, Japan) for 60 min at 95°C for antigen retrieval, then immunostained for S100 protein and isolectin 4B.

Immunohistochemistry

Sections were blocked with phosphate-buffered saline (PBS) containing 5% skim milk for 30 min at 30°C and incubated in PBS with primary antibodies, including rabbit anti-human laminin α5 (Santa Cruz Biotechnology, Santa Cruz, CA, USA; dilution, 1:20), goat anti-mouse BCAM (R&D Systems, Minneapolis, MN, USA; dilution, 1:400), and rabbit anti-S100 protein (DAKO, Glostrup, Denmark; dilution, 1:200), a marker of folliculo-stellate cells. For double immunostaining, Alexa Fluor 488-conjugated goat anti-rabbit IgG and Alexa Fluor 568-conjugated donkey anti-goat IgG (Thermo Fisher Scientific, Waltham, MA, USA) were used as secondary antibodies. Alexa Fluor 488-labeled isolectin B4 (Thermo Fisher Scientific), a marker of endothelial cells, was used for lectin histochemistry. Stained sections were subsequently coverslipped with Vectashield HardSet mounting medium with 4',6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA, USA) and observed with a confocal laser microscope (FV1000, Olympus, Tokyo, Japan). The absence of an observable nonspecific immunoreaction was confirmed by incubating sections with normal serum from either rabbit or goat, instead of the primary antibody, and then with the secondary antibody.

Real-time PCR quantification of laminin α5 chain and BCAM mRNA levels

Anterior pituitary glands were obtained from male rats at P3, P5, P10, P20, P30, and P60. Four pituitary glands were analyzed in each group. Total RNA was extracted with a RNeasy mini-kit and a RNase-free DNase set, according to the manufacturer's instructions (Qiagen, Hilden, Germany). cDNA was synthesized by using the PrimeScript RT reagent kit (Takara Bio, Shiga, Japan) with an oligo-(dT)20 primer. Quantitative real-time PCR (ABI PRISM 7900HT, Applied Biosystems, Foster City, CA,

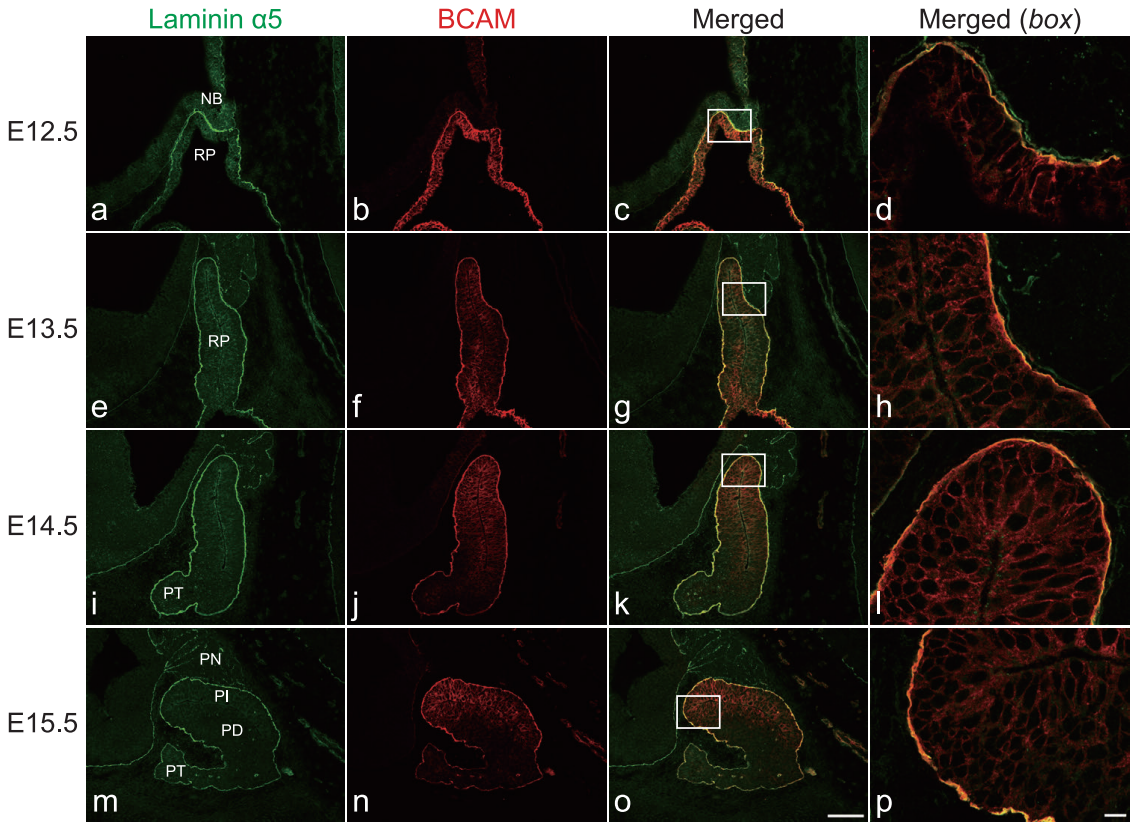

Fig. 1. Double immunohistochemistry of the laminin α5 chain and basal cell adhesion molecule (BCAM) in the developing rat pituitary gland at embryonic day 12.5 (E12.5, **a–d**), E13.5 (**e–h**), E14.5 (**i–l**), and E15.5 (**m–p**). Sagittal methanol-fixed cryosections were prepared. **a**, **e**, **i**, and **m** show laminin α5 chain immunoreactivity (green). **b**, **f**, **j**, and **n** show BCAM immunoreactivity (red). **c**, **g**, **k**, and **o** are merged images of **a** and **b**, **e** and **f**, **i** and **j**, and **m** and **n**, respectively. **d**, **h**, **l**, and **p** are high-magnification views of the *boxes* in **c**, **g**, **k**, and **o**, respectively. RP, Rathke's pouch; NB, neurohypophyseal bud; PD, pars distalis; PT, pars tuberalis; PI, pars intermedia; PN, pars nervosa. Bars = 100 μm (**a–c**, **e–g**, **i–k**, **m–o**) and 10 μm (**d**, **h**, **l**, **p**).

USA) was performed using the specific primers and SYBR Premix Ex Taq (Takara Bio) containing SYBR Green I. Primers were used to amplify laminin α5 chain cDNA fragments (GenBank accession no. NM_001191609): forward 5'-GGA TCA TGC TGA CTA CTA TGG-3' and reverse 5'-GCA GGT CTG GCA AGT AGT G-3' (161 bp) and to amplify BCAM (GenBank accession no. BC072479): forward 5'-AGT CAG CGT CGG TCT CTT G-3' and reverse 5'-GTG TGT TCT GGA CGC TCT G-3' (166 bp). For normalization of laminin α5 chain and BCAM mRNA levels, the mRNA levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH, GenBank accession no. AF106860): forward 5'-AAG GGC TCA TGA CCA CAG TC-3' and reverse 5'-GGA TGC AGG GAT GAT GTT CT-3' (116 bp) were determined. All measurements were made in duplicate, and the relative quantification was conducted using the standard curve method.

III. Results

Localization of the laminin α5 chain and BCAM in the embryonic pituitary gland

Double immunohistochemistry was used to compare the localization of the laminin α5 chain and BCAM in the embryonic pituitary gland. The laminin α5 chain was present in the basement membrane of the adenohypophyseal placode at E12.5 (Supplementary Fig. 1), and the epithelial cells of this tissue expressed BCAM (Supplementary Fig. 2). At E12.5 and E13.5, the laminin α5 chain and BCAM co-localized on the basal side of the epithelial cells of both the oral ectoderm and Rathke's pouch but not on the apical or lateral sides of the cells (Fig. 1a–h). The adenohypophysis is formed from the pars distalis, pars tuberalis, and pars intermedia. The adenohypophysis was surrounded by laminin α5 chain immunoreactivity at E14.5 (Fig. 1i). Laminin α5 chain immunoreactivity was also detected inside the pars distalis at E16.5, E17.5, E18.5, and E19.5, and immunoreactivity increased with development (Fig. 2a, e, i, m). BCAM immunoreactivity was present in the area adjacent to the adenohypophysis and on cells of both the pars intermedia and superior pars distalis at E14.5, E15.5, E16.5, E17.5, E18.5, and E19.5 (Fig. 1j, n, Fig. 2b, f, j, n). From E16.5 to E19.5, BCAM and laminin α5 chain immunoreactivity were observed inside the pars distalis. Additionally, the intensity of BCAM immunoreactivity in the pars tuberalis was weaker than that in other parts of the adenohypophysis at E15.5 and E16.5, and the immunoreactivity had mostly disappeared in the pars tuberalis at E17.5,

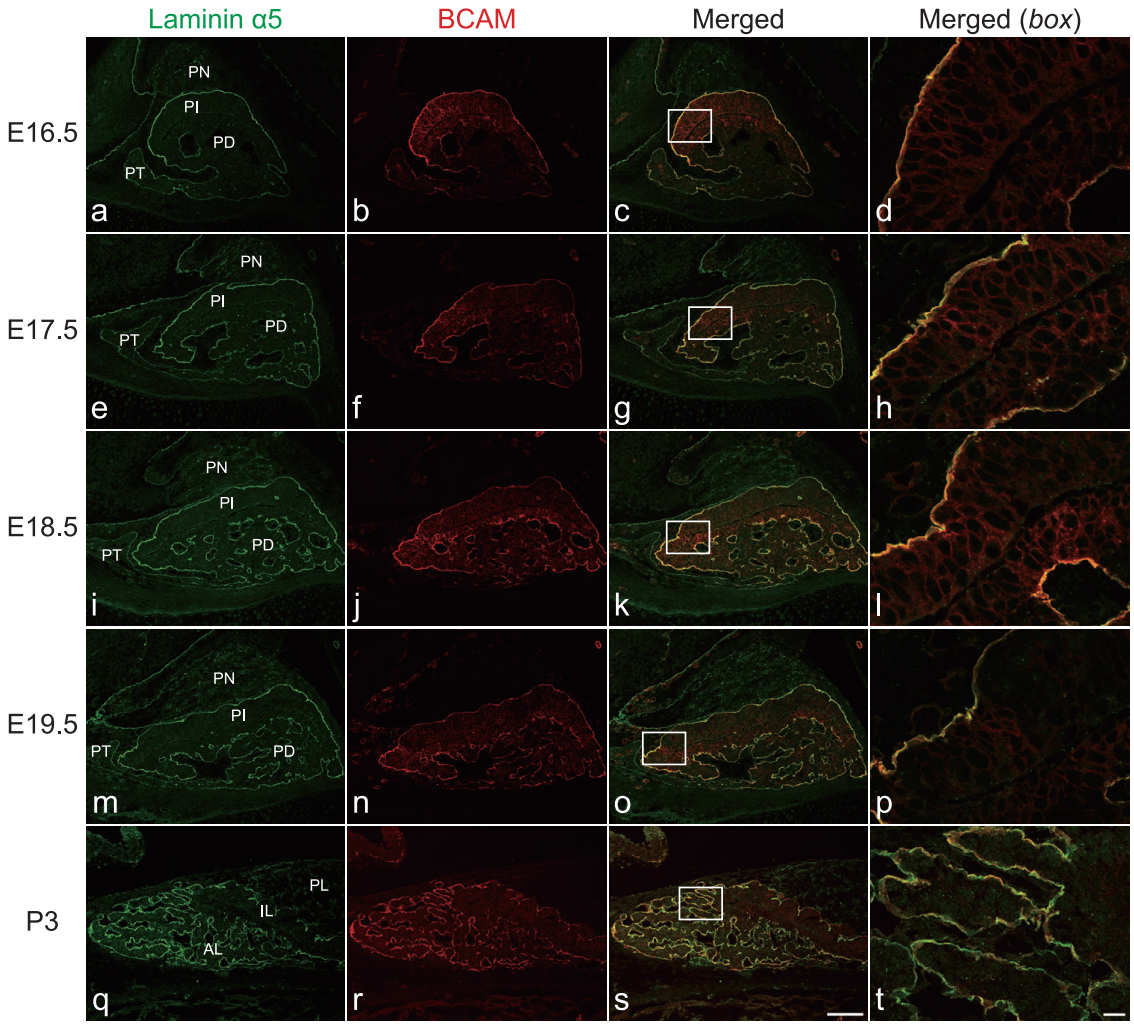

Fig. 2. Double immunohistochemistry of the laminin α5 chain and BCAM in the developing rat pituitary gland at E16.5 (**a–d**), E17.5 (**e–h**), E18.5 (**i–l**), E19.5 (**m–p**), and postnatal day 3 (P3, **q–t**). Sagittal (E16.5–19.5) and frontal (P3) methanol-fixed cryosections were prepared. **a**, **e**, **i**, **m**, and **q** show laminin α5 chain immunoreactivity (green). **b**, **f**, **j**, **n**, and **r** show BCAM immunoreactivity (red). **c**, **g**, **k**, **o**, and **s** are merged images of **a** and **b**, **e** and **f**, **i** and **j**, **m** and **n**, and **q** and **r**, respectively. **d**, **h**, **l**, **p**, and **t** are high-magnification views of the *boxes* in **c**, **g**, **k**, **o**, and **s**, respectively. PD, pars distalis; PT, pars tuberalis; PI, pars intermedia; PN, pars nervosa; AL, anterior lobe; IL, intermediate lobe; PL, posterior lobe. Bars = 100 μm (**a–c**, **e–g**, **i–k**, **m–o**, **q–s**) and 10 μm (**d**, **h**, **l**, **p**, **t**).

E18.5, and E19.5. The laminin α5 chain and BCAM co-localized in the area surrounding the adenohypophysis at E14.5, E15.5, E16.5, E17.5, E18.5, and E19.5 and inside the pars distalis at E16.5, E17.5, E18.5, and E19.5 (Fig. 1k, l, o, p, Fig. 2c, d, g, h, k, l, o, p). In the neurohypophysis (neurohypophyseal bud or pars nervosa), only laminin α5 chain immunoreactivity was observed.

Postnatal expression of the laminin α5 chain and BCAM in the rat anterior pituitary

Both laminin α5 chain and BCAM immunoreactivity were observed in the rat anterior pituitary gland at P3, and the signals were partially co-localized (Fig. 2q–t). Real-time PCR was used to determine the levels of transcripts for the laminin α5 chain and BCAM present in the glands at postnatal stages. The relative levels of laminin α5 chain mRNA in the rat anterior pituitary gland gradually decreased throughout growth (Fig. 3a), as did expression levels of BCAM mRNA (Fig. 3b). In quantitative analysis, both transcripts were detectable at P60.

Immunohistochemical techniques were used to confirm the existence of the laminin α5 chain and BCAM in the anterior pituitary gland of the adult rat. Double immunohistochemistry revealed that the laminin α5 chain and BCAM were present in the anterior, intermediate, and posterior lobes at P60 (Fig. 4a–c). In the anterior lobe, laminin α5 chain and BCAM immunoreactivity were located in the area surrounding blood vessels and in the parenchyma (Fig. 4d, e). The laminin α5 chain and BCAM were partially co-localized in the gland (Fig. 4f).

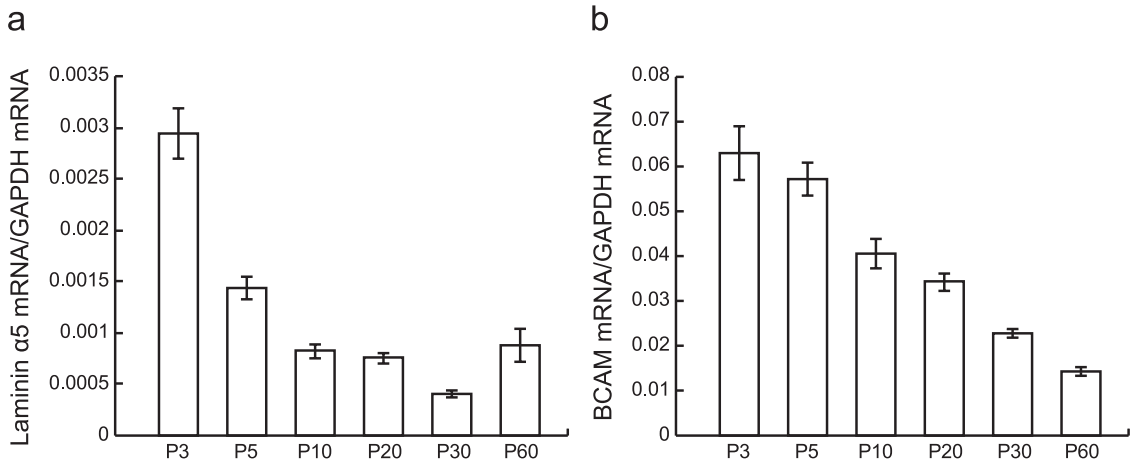

Fig. 3. Postnatal changes in laminin α5 chain and BCAM mRNA levels in the rat anterior pituitary gland, as determined by real-time PCR. Laminin α5 chain and BCAM mRNA levels at P3, P5, P10, P20, P30, and P60 were normalized to the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA level as an internal control. Four animals were used for each analysis at each stage. Data are expressed as mean ± SEM (n = 4).

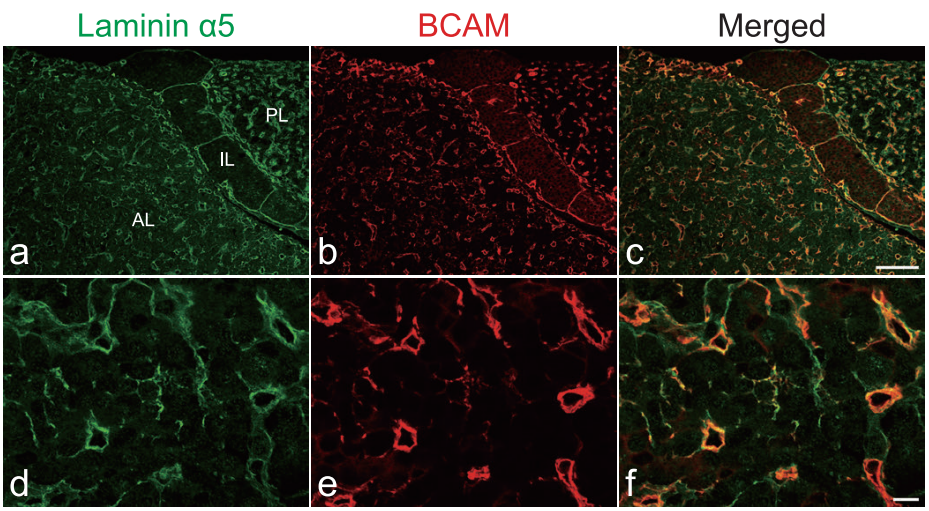

Fig. 4. Double immunohistochemistry of the laminin α5 chain and BCAM in the rat pituitary gland at P60. Frontal-plane methanol-fixed cryosections were prepared. **a** and **d** show laminin α5 chain immunoreactivity (green). **b** and **e** show BCAM immunoreactivity (red). **c** and **f** are merged images of **a** and **b**, and **d** and **e** respectively. **d–f** are high-magnification views of the anterior lobe (AL). IL, intermediate lobe; PL, posterior lobe. Bars = 100 μm (**a–c**), 10 μm (**d–f**).

Characterization of BCAM-expressing cells in the anterior pituitary gland

Double immunohistochemistry was used to identify cells expressing BCAM. BCAM immunoreactivity in the parenchyma of the anterior lobe was located on folliculo-stellate cells, which were identified by immunohistochemistry for S100 protein (Fig. 5a–c). BCAM immunoreactivity around blood vessels of the anterior lobe was located on endothelial cells, which were identified by immunohistochemistry for isolectin B4 (Fig. 5d–f). BCAM immunoreactivity was not detected in any type of endocrine cell (Supplementary Fig. 3).

IV. Discussion

This is the first report to describe the distribution of the laminin α5 chain and its specific receptor, BCAM, in the rat pituitary gland. Immunohistochemical analysis showed that the laminin α5 chain is co-localized with BCAM in the gland throughout tissue development. In addition, folliculo-stellate cells and endothelial cells express BCAM in the adult anterior pituitary gland.

Rathke's pouch is composed of undifferentiated cells, which are committed to become the adenohypophysis through cell proliferation and differentiation. This tissue is surrounded by a basement membrane. Our immunohisto-

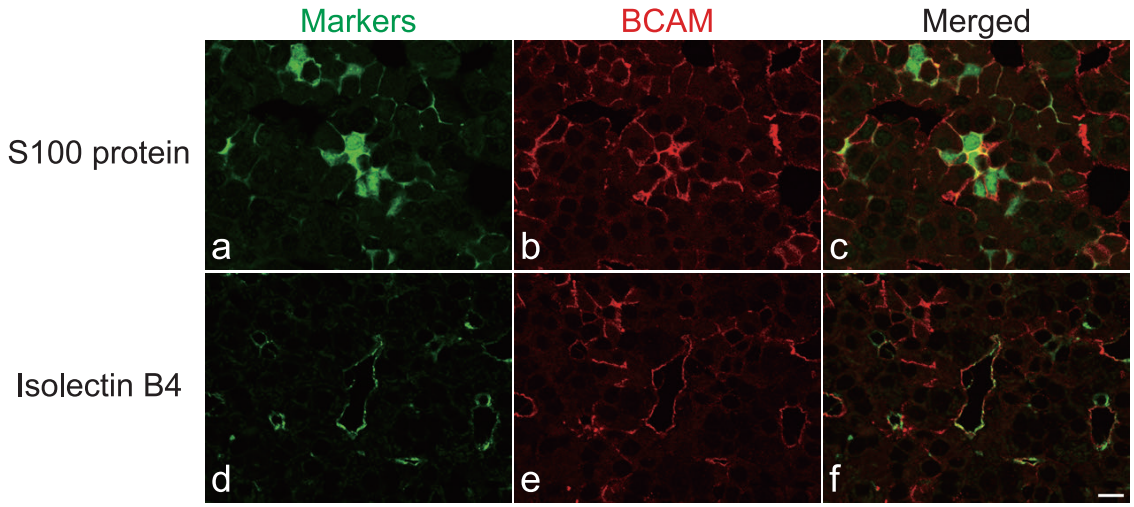

Fig. 5. BCAM-expressing cells in the rat anterior pituitary gland at P60. **a–c**: Double immunostaining of S100 protein (**a**, a marker of folliculo-stellate cells, green) and BCAM (**b**, red) in the anterior pituitary gland. **d–f**: Double immunostaining of isolectin B4 (**d**, a marker of endothelial cells, green) and BCAM (**e**, red) in the anterior pituitary gland. Bar = 10 μm (**a–f**).

chemical study showed that laminins containing the α5 chain are distributed in the basement membrane of the embryonic adenohypophysis, including Rathke's pouch (Supplementary Fig. 1, Fig. 1a, e, i, m, Fig. 2a, e, i, m). This distribution pattern is similar to those reported for both type IV collagen and laminin [2, 10].

BCAM, also known as the Lutheran blood glycoprotein, is a membrane protein in the immunoglobulin superfamily. It binds to the LG domains of the laminin α5 chain and is a specific receptor for laminins containing that chain [7, 17, 25]. In the present study, co-localization of BCAM and the laminin α5 chain suggested that the BCAM of epithelial cells in the primordial adenohypophysis functions as a receptor for this laminin chain (Figs. 1, 2). Our previous study reported that the α5 chain is produced in epithelial cells of the primordial adenohypophysis [21]. These findings suggest that cells of the primordial adenohypophysis respond in an autocrine manner to endogenously produced laminin α5 chain via BCAM and that this signal may contribute to basement membrane development and maintenance. In contrast, the embryonic neurohypophysis exhibited only laminin α5 chain immunoreactivity, and the disappearance of BCAM immunoreactivity in the pars tuberalis of adenohypophysis at E16.5, E17.5, E18.5, and E19.5 suggests either that laminin α5 chain-mediated signaling via BCAM is not involved in neurohypophysis and pars tuberalis development or cells in this region have other receptors for the chain.

Our observation of laminin α5 chain and BCAM immunoreactivity inside the gland from E16.5 to E19.5 is similar to that regarding the reported location of capillaries (Fig. 2a–p). After E16.5, capillaries invade the rat pars distalis from circumferential interstitial tissue [8]. In addition, a study using conditional knock-out mice found that integrin β1, a common receptor of several extracellular matrix molecules, including laminin, is required for developmental angiogenesis in the pituitary gland [22]. Regarding the mechanism, BCAM appeared to regulate cell migration by competitively modulating integrin binding to the laminin α5 chain in a human fibrosarcoma cell line [18]. Past and present evidence suggests that BCAM may regulate the integrin signal involved in capillary invasion of the pars distalis.

Our previous study using *in situ* hybridization revealed that laminin α5 chain mRNA is also expressed in the cells of the rat anterior pituitary gland at P5, P10, and P30 [21]. In the present study, quantitative analysis for laminin α5 mRNA and immunohistochemical analysis revealed that laminin α5 is present in the gland postnatally, including in adult rats at P60 (Fig. 2q, Fig. 3a, Fig, 4a, d) and that its distribution is similar to the localization of laminin in the gland, as previously reported [9, 24]. To better understand how the amount of the laminin α5 chain is controlled, future studies should attempt to identify both the cells producing it in the gland and the systems that regulate its expression.

This study additionally revealed postnatal BCAM expression in the rat anterior pituitary gland. Furthermore, we found evidence that these receptor-expressing cells may contact the basement membrane containing the laminin α5 chain, as indicated by co-localization of the immunoreactivity (Fig. 2q–t, Fig. 3b, Fig. 4). BCAM is expressed in human dermal keratinocytes and murine glomerular endothelium [3, 16]. In the adult rat anterior pituitary gland, folliculo-stellate cells and endothelial cells expressed BCAM (Fig. 5). We previously reported that folliculo-stellate cell functions are influenced by the interaction between the cell and laminin via integrin β1 [12, 13]. Integrin β1 expressed in endothelial cells is involved in angiogenesis [19]. These past and present results suggest that

BCAM may contribute to the functions of both folliculo-stellate cells and endothelial cells by regulating integrin signaling in the adult rat anterior pituitary gland.

Our previous studies showed that laminin α chains other than α5, including α1 through α4, are expressed during gland development [20, 21], but their distributions and the functional differences between them are unclear. In addition, various laminin receptors, including integrins and syndecans, may be involved in regulating anterior pituitary cell function [11, 14, 15]. Future studies should attempt to clarify the functions of these laminin α chains and their receptors in the gland.

V. Conflicts of Interest

The authors have no conflicts of interest that might prejudice the impartiality of this research.

VI. Acknowledgments

We thank David Kipler, ELS, of Supernatant Communications for assistance in manuscript preparation.

This work was supported in part by JSPS KAKENHI Grants (Numbers 16K18981 to MA, 26460281 to KF), by promotional funds from the Keirin Race of the Japan Keirin Association, and by the Jichi Medical University Young Investigator Award from Jichi Medical University School of Medicine.

VII. References

1. Aumailley, M., Bruckner-Tuderman, L., Carter, W. G., Deutzmann, R., Edgar, D., Ekblom, P., Engel, J., Engvall, E., Hohenester, E., Jones, J. C., Kleinman, H. K., Marinkovich, M. P., Martin, G. R., Mayer, U., Meneguzzi, G., Miner, J. H., Miyazaki, K., Patarroyo, M., Paulsson, M., Quaranta, V., Sanes, J. R., Sasaki, T., Sekiguchi, K., Sorokin, L. M., Talts, J. F., Tryggvason, K., Uitto, J., Virtanen, I., von der Mark, K., Wewer, U. M., Yamada, Y. and Yurchenco, P. D. (2005) A simplified laminin nomenclature. *Matrix Biol.* 24; 326–332.
2. Berardi, M., Hindelang, C., Félix, J. M. and Stoeckel, M. E. (1999) L1 and laminin: their expression during rat hypophysis ontogenesis and in adult neurohemal areas. *Int. J. Dev. Neurosci.* 17; 121–130.
3. Bernemann, T. M., Podda, M., Wolter, M. and Boehncke, W. H. (2000) Expression of the basal cell adhesion molecule (B-CAM) in normal and diseased human skin. *J. Cutan. Pathol.* 27; 108–111.
4. Cheng, Y. S., Champliaud, M. F., Burgeson, R. E., Marinkovich, M. P. and Yurchenco, P. D. (1997) Self-assembly of laminin isoforms. *J. Biol. Chem.* 272; 31525–31532.
5. Domogatskaya, A., Rodin, S. and Tryggvason, K. (2008) Functional diversity of laminins. *Annu. Rev. Cell Dev. Biol.* 28; 523–553.
6. Durbeej, M. (2010) Laminins. *Cell Tissue Res.* 339; 259–268.
7. El Nemer, W., Gane, P., Colin, Y., Bony, V., Rahuel, C., Galactéros, F., Cartron, J. P. and Le Van Kim, C. (1998) The Lutheran blood group glycoproteins, the erythroid receptors for laminin, are adhesion molecules. *J. Biol. Chem.* 273; 16686–16693.
8. Higuchi, M., Kato, T., Yoshida, S., Ueharu, H., Nishimura, N. and Kato, Y. (2015) PRRX1- and PRRX2-positive mesenchymal stem/progenitor cells are involved in vasculogenesis during rat embryonic pituitary development. *Cell Tissue Res.* 361; 557–565.
9. Holck, S., Albrechtsen, R. and Wewer, U. M. (1987) Laminin in the anterior pituitary gland of the rat. Laminin in the gonadotrophic cells correlates with their functional state. *Lab. Invest.* 56; 481–488.
10. Horacek, M. J., Thompson, J. C., Dada, M. O. and Terracio, L. (1993) The extracellular matrix components laminin, fibronectin, and collagen IV are present among the epithelial cells forming Rathke's pouch. *Acta Anat. (Basel)* 147; 69–74.
11. Horacek, M. J., Kawaguchi, T. and Terracio, L. (1994) Adult adenohypophysial cells express beta 1 integrins and prefer laminin during cell-substratum adhesion. *In Vitro Cell. Dev. Biol. Anim.* 30A; 35–40.
12. Horiguchi, K., Kikuchi, M., Kusumoto, K., Fujiwara, K., Kouki, T., Kawanishi, K. and Yashiro, T. (2010) Living-cell imaging of transgenic rat anterior pituitary cells in primary culture reveals novel characteristics of folliculo-stellate cells. *J. Endocrinol.* 204; 115–123.
13. Horiguchi, K., Fujiwara, K., Ilmiawati, C., Kikuchi, M., Tsukada, T., Kouki, T. and Yashiro, T. (2011) Caveolin 3-mediated integrin β1 signaling is required for the proliferation of folliculostellate cells in rat anterior pituitary gland under the influence of extracellular matrix. *J. Endocrinol.* 210; 29–36.
14. Horiguchi, K., Kouki, T., Fujiwara, K., Tsukada, T., Ly, F., Kikuchi, M. and Yashiro, T. (2012) 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. *J. Endocrinol.* 214; 199–206.
15. Horiguchi, K., Syaidah, R., Fujiwara, K., Tsukada, T., Ramadhani, D., Jindatip, D., Kikuchi, M. and Yashiro, T. (2013) Expression of the cell-surface heparan sulfate proteoglycan syndecan-2 in developing rat anterior pituitary gland. *Cell Tissue Res.* 353; 473–481.
16. Huang, J., Filipe, A., Rahuel, C., Bonnin, P., Mesnard, L., Guérin, C., Wang, Y., Le Van Kim, C., Colin, Y. and Tharaux, P. L. (2014) Lutheran/basal cell adhesion molecule accelerates progression of crescentic glomerulonephritis in mice. *Kidney Int.* 85; 1123–1136.
17. Kikkawa, Y., Moulson, C. L., Virtanen, I. and Miner, J. H. (2002) Identification of the binding site for the Lutheran blood group glycoprotein on laminin alpha 5 through expression of chimeric laminin chains in vivo. *J. Biol. Chem.* 277; 44864–44869.
18. Kikkawa, Y., Ogawa, T., Sudo, R., Yamada, Y., Katagiri, F., Hozumi, K., Nomizu, M. and Miner, J. H. (2013) The lutheran/basal cell adhesion molecule promotes tumor cell migration by modulating integrin-mediated cell attachment to laminin-511 protein. *J. Biol. Chem.* 288; 30990–31001.
19. Malan, D., Wenzel, D., Schmidt, A., Geisen, C., Raible, A., Bölck, B., Fleischmann, B. K. and Bloch, W. (2010) Endothelial beta1 integrins regulate sprouting and network formation during vascular development. *Development* 137; 993–1002.
20. Ramadhani, D., Tsukada, T., Fujiwara, K., Horiguchi, K., Kikuchi, M. and Yashiro, T. (2012) Laminin isoforms and laminin-producing cells in rat anterior pituitary. *Acta Histochem. Cytochem.* 45; 309–315.
21. Ramadhani, D., Tsukada, T., Fujiwara, K., Azuma, M., Kikuchi, M. and Yashiro, T. (2014) Changes in laminin chain expression in pre- and postnatal rat pituitary gland. *Acta Histochem. Cytochem.* 47; 231–237.
22. Scully, K. M., Skowronska-Krawczyk, D., Krawczyk, M.,

Merkurjev, D., Taylor, H., Livolsi, A., Tollkuhn, J., Stan, R. V. and Rosenfeld, M. G. (2016) Epithelial cell integrin β1 is required for developmental angiogenesis in the pituitary gland. *Proc. Natl. Acad. Sci. U S A* 113; 13408–13413.

23. Spenlé, C., Simon-Assmann, P., Orend, G. and Miner, J. H. (2013) Laminin α5 guides tissue patterning and organogenesis. *Cell Adh. Migr.* 7; 90–100.
24. Tougard, C., Louvard, D., Picart, R. and Tixier-Vidal, A. (1985) Immunocytochemical localization of laminin in rat anterior pituitary cells in vivo and in vitro. *In Vitro Cell. Dev. Biol.* 21; 57–61.
25. Udani, M., Zen, Q., Cottman, M., Leonard, N., Jefferson, S., Daymont, C., Truskey, G. and Telen, M. J. (1998) Basal cell adhesion molecule/lutheran protein. The receptor critical for sickle cell adhesion to laminin. *J. Clin. Invest.* 101; 2550–2558.
26. Yurchenco, P. D. (2011) Basement membranes: cell scaffoldings and signaling platforms. *Cold Spring Harb. Perspect. Biol.* 3; a004911.