JSHC
Japan Society of Histochemistry and Cytochemistry
Established 1959

Acta Histochem. Cytochem. 45 (5): 309–315, 2012
doi:10.1267/ahc.12028

Laminin Isoforms and Laminin-Producing Cells in Rat Anterior Pituitary

Dini Ramadhani[1], Takehiro Tsukada[1], Ken Fujiwara[1], Kotaro Horiguchi[1], Motoshi Kikuchi[1] and Takashi Yashiro[1]

[1]*Division of Histology and Cell Biology, Department of Anatomy, Jichi Medical University School of Medicine, Tochigi, Japan*

Received July 24, 2012; accepted August 7, 2012; published online October 12, 2012

Laminin is a key component of the basement membrane and is involved in the structural scaffold and in cell proliferation and differentiation. Research has identified 19 laminin isoforms, which are assemblies of α, β, and γ chains (eg, the α1, β1, and γ1 chains form the laminin 111 isoform). Although laminin is known to be present in the anterior pituitary, the specific laminin isoforms have not been identified. This study used molecular biological and histochemical techniques—namely, RT-PCR, immunohistochemistry, and *in situ* hybridization—to identify the laminin isoforms and laminin-producing cells in rat anterior pituitary. RT-PCR showed that laminin α1, α3, and α4 genes were expressed in anterior pituitary. Immunohistochemistry revealed laminin α1 in gonadotrophs and laminin α4 in almost all vascular endothelial cells. Laminin α3 was seen in a subset of vascular endothelial cells. We then performed *in situ* hybridization to localize β and γ chains in these cells and found that laminin β1, β2, and γ1 were expressed in gonadotrophs and that laminin β1 and γ1 were expressed in endothelial cells. In conclusion, we identified gonadotroph-type (laminin 111 and 121) and vascular-type (laminin 411 and 311) laminin isoforms in rat anterior pituitary.

Key words: anterior pituitary, laminin, basement membrane, gonadotroph, endothelial cell

I. Introduction

The basement membrane (BM) is a specialized, 50- to 100-nm thick, extracellular matrix that surrounds every cell cord including endothelial cells. It has a role in the structural support and barriers of tissues, and in the proliferation, adhesion, migration, and differentiation of cells. There are 4 major components of BM: laminin, type IV collagen, nidogen, and heparan-sulphate proteoglycan core protein [1, 2, 25]. Laminin is required for BM assembly, as the N-terminal (domain VI) of laminin promotes laminin polymerization [1, 2, 17]. Laminin is also important in regulating cellular behavior and function through its interaction with cell surface receptors such as integrin and dystroglycan [5, 15, 17].

Laminin is a cross-shaped heterotrimeric glycoprotein comprising single α, β, and γ chains [2]. The three chains form a complex by disulfide bounds, and the complex is secreted as matrices after glycosylation. Thus far, research has identified 5 α, 3 β, and 3 γ chains. By combining into heterotrimers, they assemble to form 19 different laminin isoforms (eg, laminin 111 comprises the α1, β1, and γ1 chains) [5]. Each laminin isoform is synthesized by numerous cell types and has distinct signals in a variety of tissues [8]. For instance, laminin 111 induces cell differentiation in mammary gland cells [21], while laminin 411 mediates adhesion and migration of platelets, leukocytes, and vascular endothelial cells [6]. Thus, laminin isoforms must be identified so as to reveal their functional roles.

In contrast to other tissues, the specific laminin isoforms expressed in the anterior pituitary have not been identified, although several studies have confirmed the presence of laminin protein in this gland [10, 11, 13, 19]. In the present study, we used RT-PCR to identify laminin chains expressed in the anterior pituitary. In addition, we used immunohistochemistry and *in situ* hybridization to identify laminin isoforms and laminin-producing cells.

Correspondence to: Takashi Yashiro, MD, PhD, Department of Anatomy, Jichi Medical University School of Medicine, 3311–1 Yaskushiji, Shimotsuke, Tochigi 329–0498, Japan.
E-mail: tyashiro@jichi.ac.jp

II. Materials and Methods

Animals

Wistar rats were purchased from Japan SLC (Shizuoka, Japan). Transgenic S100β-GFP rats, which express GFP in FS cells under the control of exogenous S100β protein gene promoter, were kindly donated by Prof. K. Inoue of Saitama University and bred in our animal facility [12]. Animals were given conventional food and water *ad libitum* and were kept under a 12 hr light/12 hr dark cycle. All animal experiments were performed after receiving approval from the Institutional Animal Experiment Committee of the Jichi Medical University and were conducted in accordance with the Institutional Regulations for Animal Experiments and Fundamental Guideline for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions, under the jurisdiction of the Japanese Ministry of Education, Culture, Sports, Science and Technology.

Tissue preparation

Animals (postnatal day 60) weighing 250–300 g were deeply anesthetized intraperitoneally with pentobarbital sodium (Kyoritsu Seiyaku, Tokyo, Japan). A solution of 4% paraformaldehyde in 0.05 M phosphate buffer (PB), pH 7.4, was perfused through the left ventricle. Pituitaries were dissected out and immersed overnight in the same fixative at 4°C. The tissues were transferred into 30% sucrose in 0.05 M PB buffer, pH 7.2, at 4°C for 2 days. The tissues were then embedded in Tissue-Tek compound (Sakura Finetechnical, Tokyo, Japan) and frozen rapidly. A cryostat (CM3000; Leica Microsystems, Wetzlar, Germany) was used to make frontal sections (8 μm), which were used for immunohistochemistry and *in situ* hybridization.

Reverse transcription-polymerase chain reaction (RT-PCR)

Anterior pituitaries were dissected and rapidly immersed in liquid nitrogen. Trizol RNA extraction (Qiagen, Hilden, Germany) and subsequent RNase-free DNase I treatment (Promega, Madison, WI, USA) were performed to extract total RNA according to the manufacturer's instructions. cDNA was made by using the SuperScript III Reverse Transcriptase kit with oligo-d$(T)_{20}$ primer (Life Technologies, CA, USA). Gene expression of laminin chains was determined by PCR using specific primers (Table 1). The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control.

Table 1. *Primers for RT-PCR/cRNA probes*

No	Primer		Forward sequence (5'-3')	Reverse sequence (5'-3')
1	laminin α1 (594 bp)	GenBank: NM_001108237	CGTCATGCAGATGTCCAAAC	GTCTCCTGGACTGTGGCATT
2	laminin α2 (568 bp)	GenBank: XM_219866	TACCAAAGGCATTGCTTTCC	TGCACACGTCTCACAAGACA
3	laminin α3 (678 bp)	GenBank: NM_173306	GGATGCCTCCAAGGACTACA	TTCTCTTTGGGTGGCTCTG
4	laminin α4 (560 bp)	GenBank: NM_001107635	GACACGTGACCGACATGAAC	GGCCTGGTTAATGGATTCCT
5	laminin α5 (622 bp)	GenBank: NM_001191609	GAAGCTGGCTCTTGTCATCC	GAAAGAGGTCAGGGGAGGTC
6	laminin β1 (589 bp)	GenBank: NM_001106721	GAGCGGTCTTCTGACTTTGG	CAAAGTGACACGAGCTGGAA
7	laminin β2 (666 bp)	GenBank: NM_012974	CCTTGGATGTACCTGGCTGT	TGTCTCCCAGTGTGTGAAGC
8	laminin β3 (692 bp)	GenBank: NM_001100841	GGTTGCTAGATGCCAAGAGC	CCTGATCATTTGCCTGGTCT
9	laminin γ1 (650 bp)	GenBank: NM_053966	AAGTGCCTGCCTTTCTTCAA	GAGCCATCTCTCTGCTCCAC
10	laminin γ2 (639 bp)	GenBank: NM_001100640	GGCAGAGCCTGTCTTTTGAC	CCTCCTCTGTCTCAGGCATC
11	laminin γ3 (597 bp)	GenBank: NM_001107830	CGAAGACCCTGCTAGCTGAC	CCTCCAGCTGCCTTAGTTTG

Table 2. *Primary antibodies*

Antigen	Immunized animal	Dilution factor	Supplier
mouse laminin (LSL-LB-1013)	Rabbit	1:1600	Cosmo Bio, Tokyo, Japan
human laminin α1 (H-300)	Rabbit	1:25	Santa Cruz Biotechnology, Santa Cruz, CA
mouse laminin α3 (M-20)	Goat	1:25	Santa Cruz Biotechnology
human laminin α4 (V-20)	Goat	1:25	Santa Cruz Biotechnology
human LHβ	Goat	1:100	Santa Cruz Biotechnology
rat GH	Goat	1:400	R&D Systems, Minneapolis, MN
human TSHβ	Mouse	1:800	Merck KGaA, Darmstadt, Germany
synthetic PRL	Mouse	1:800	QED Bioscience, San Diego, CA
human 17-39-ACTH	Mouse	1:1600	EMD Millipore, Billerica, MA
human desmin	Mouse	1:100	DAKO, Glostrup, Denmark
ovine LHβ	Rabbit	1:6400	Advance, Tokyo, Japan

GH, growth hormone; LHβ, luteinizing hormone β subunit; TSHβ, thyroid-stimulating hormone β subunit; PRL, prolactin hormone; ACTH, adrenocorticotropic hormone.

Immunofluorescence

Immunofluorescence was performed as previously described with some modifications [7]. Briefly, sections were heat-retrieved with 0.01 M citrate buffer, pH 6.0, in 90°C for 10 min [16]. The sections were then incubated in primary antibodies (Table 2) for 90 min at 30°C. If necessary, biotinylated isolectin B4 (B-1205; Vector Laboratories, CA, USA), which binds to galactose residue on the surface of endothelial cells [3], was added to the primary antibodies to detect endothelial cells. The sections were then incubated in secondary antibodies, namely, Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:200) and Alexa Fluor 568-conjugated goat anti-rabbit IgG (1:200) (Life Technologies). Biotinylated isolectin B4 was detected by Alexa Fluor 488-conjugated streptavidin (1:400) or Alexa Fluor 568-conjugated streptavidin (1:400) (Life Technologies). Sections were scanned using a confocal laser microscope (FV1000; Olympus, Tokyo, Japan).

In situ hybridization

Because suitable commercial anti-laminin β- and γ-chain antibodies for immunohistochemistry could not be obtained, we used *in situ* hybridization to determine the localization of the laminin β and γ chains. *In situ* hybridization was performed as described previously [7]. Briefly, to make complementary RNA (cRNA), DNA fragments were amplified from cDNA of adult rat pituitary, using gene-specific primers (Table 1). DNA fragments were ligated into the pGEM-T vector (Promega) and cloned. Gene-specific antisense or sense digoxigenin (DIG)-labeled cRNA probes were made by using a Roche DIG RNA labeling kit (Roche Diagnostics, Penzberg, Germany). DIG-labeled cRNA probe hybridization was performed at 55°C for 16–18 hr. Each type of mRNA was visualized with alkaline phosphatase-conjugated anti-DIG antibody (Roche Diagnostics) by using 4-nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate (Roche Diagnostics). Sections treated with DIG-labeled sense RNA probes were used as negative control.

The *in situ* hybridization sections were then immunohistochemically stained as described previously [7]. Briefly, the sections were incubated with rabbit polyclonal anti-luteinizing hormone β antibody or biotinylated isolectin B4 (an endothelial cell marker) for 90 min at 30°C (for details, see Table 2). The immunoreactive signal was detected by using a Vecstain ABC kit (Vector Laboratories) and 3,3'-diaminobenzidine (Dojindo Laboratories, Kumamoto, Japan). Stained sections were observed using an AX-80 microscope (Olympus).

III. Results

Immunohistochemistry of laminin and isolectin B4

To localize laminin deposition in anterior pituitary gland, pituitary sections were stained using anti-laminin antibody (LSL-LB-1013), which recognizes native form laminin protein and is not laminin chain-specific antibody (Fig. 1a). To observe vascular localization, we also stained endothelial cells with biotinylated isolectin B4. Capillary walls stained by isolectin B4 were distributed throughout the anterior pituitary (Fig. 1b). Laminin was deposited beneath the capillaries (vascular basement membrane) and interstitial spaces of the anterior pituitary (Fig. 1a–d). Laminin was also observed in the cytoplasm of some pituitary cells (Fig. 1a–d).

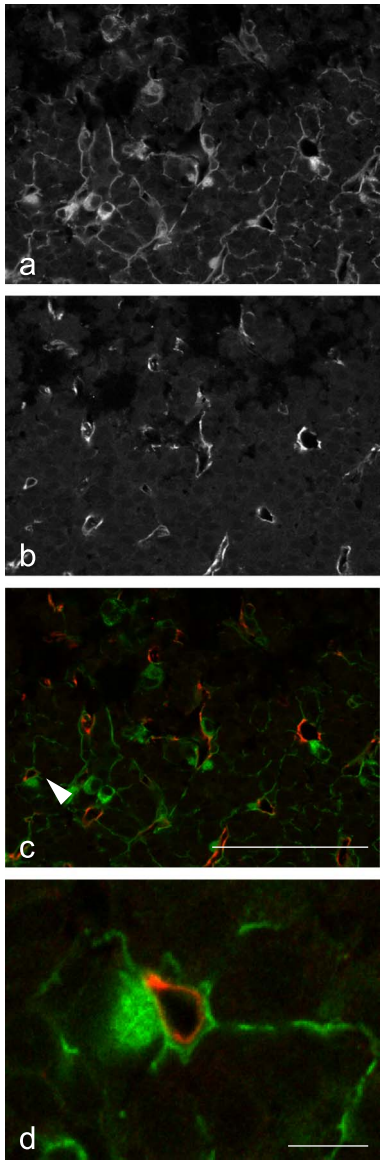

Fig. 1. Distribution of laminin and endothelial cells in rat anterior pituitary. Immunofluorescence of anti-laminin antibody (LSL-LB-1013) and biotinylated isolectin B4 (an endothelial cell marker) are seen in **a** and **b**, respectively. A merged image is shown in **c** (laminin, green; isolectin B4, red). Bars=100 μm. Higher magnification of the merged image (arrowhead area in **c**) is shown in **d**. Bar=10 μm. Laminin protein was expressed in the vascular basement membrane, interstitial spaces, and a subset of pituitary cells.

Expression of laminin chains

Expression of laminin chains in the anterior pituitary was determined by RT-PCR. Laminin α1, α3, α4, β1, β2, and γ1 were expressed, while laminin α2, α5, β3, γ2, γ3 were weakly expressed (Fig. 2).

Because transcripts of laminin α1, α3, and α4 chains were detected in the gland, we stained these chains using α chain-specific antibodies. Laminin α1 was detected only in the cytoplasm of some pituitary cells; it was not colocalized with isolectin B4 (Fig. 3a–d). Strong immunostaining of both laminin α3 and laminin α4 was detected only in endothelial cells. Laminin α3 staining showed a punctate pattern in cytoplasm and the cell membrane, and its distribution was very limited (Fig. 3e–h). In comparison, laminin α4 staining was diffuse in endothelial cell cytoplasm and some endothelial cell membranes, and almost all endothelial cells were stained with laminin α4 (Fig. 3i–l).

To characterize the cells that produced laminin α1, laminin α1-immunopositive cells were detected by hormones and S100β (a marker of folliculo-stellate cells). Laminin α1-immunopositive cells were colocalized only with LHβ-immunopositive cells (gonadotrophs) (Fig. 4a–g).

We performed *in situ* hybridization of laminin β and γ chains to identify the laminin isoforms expressed in gonadotrophs and endothelial cells. Laminin β1, β2, and γ1 were expressed in gonadotrophs (Fig. 5a–c), and laminin β1 and γ1 were expressed in isolectin B4-positive cells (endothelial cells) (Fig. 5d–f).

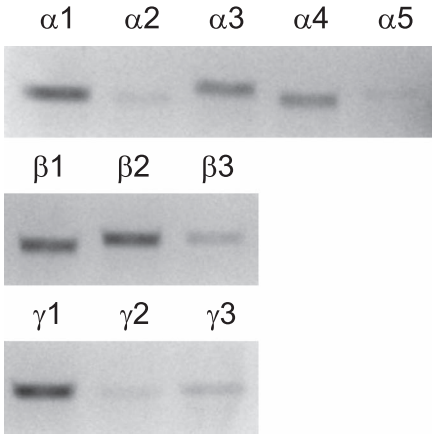

Fig. 2. Gene expression of laminin chains as determined by RT-PCR. The total mRNA fraction extracted from rat anterior pituitary was analyzed using laminin chain-specific primers. Laminin α1, α3, α4, β1, β2, and γ1 were expressed in the anterior pituitary.

IV. Discussion

In this study, we used molecular biological and histochemical approaches to identify laminin chains and

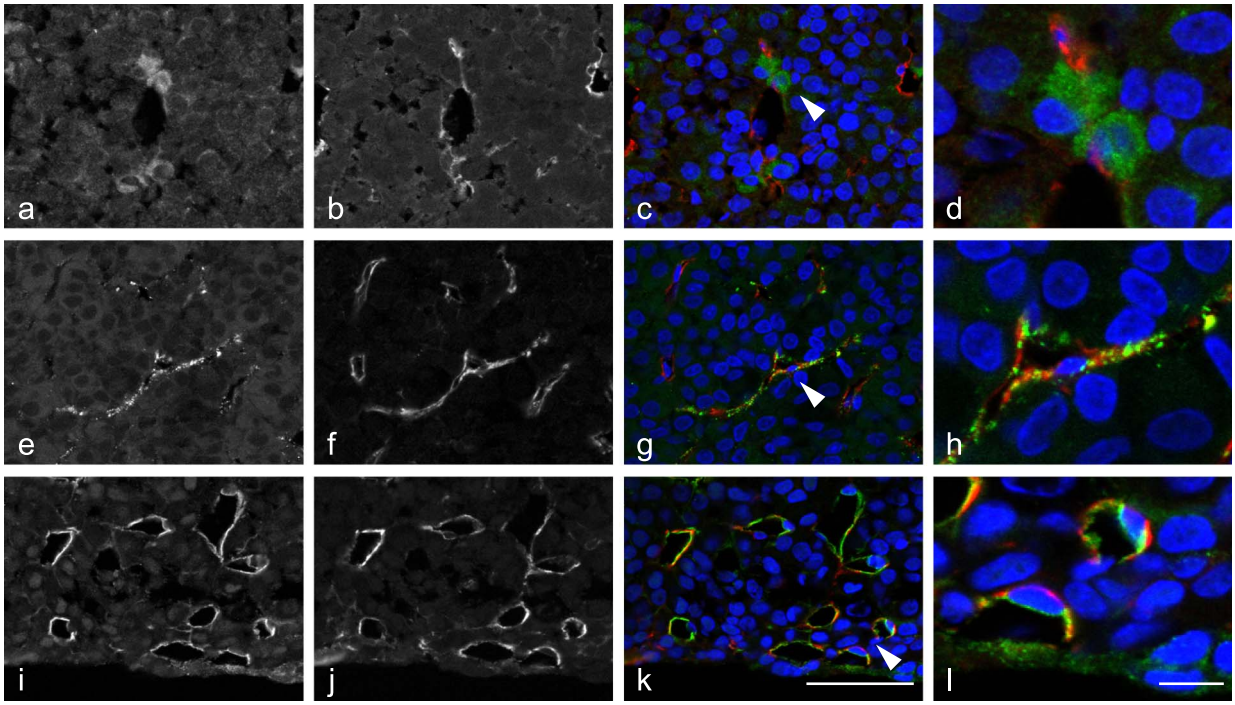

Fig. 3. Localization of laminin α chains in rat anterior pituitary. Immunofluorescence of laminin α1, α3, and α4 are shown in **a**, **e**, and **i**, respectively. Biotinylated isolectin B4 (**b, f, j**) was used as an endothelial cell marker. Merged images are shown in **c**, **g**, and **k** (laminin chains, green; isolectin B4, red; DAPI, blue; colocalization, yellow). Bar=50 μm. Higher magnification views of the merged images (arrowheads in **c, g, k**) are shown in **d**, **h**, and **l**. Bar=10 μm. DAPI was used as a nucleus marker. Laminin α1 was detected in a subset of pituitary cells. Laminin α3 deposition was observed in endothelial cells, although its distribution was very limited. Laminin α4 was detected in almost all endothelial cells.

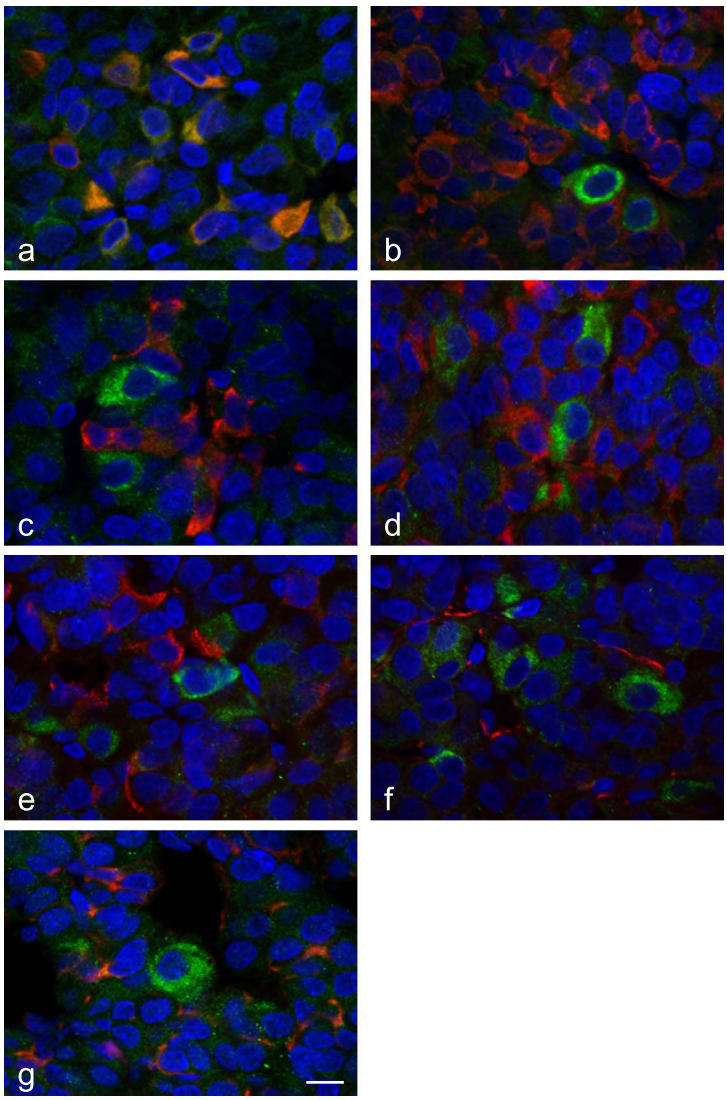

Fig. 4. Laminin α1-positive cells in rat anterior pituitary. Immunofluorescence of laminin α1 (green) and anterior pituitary hormones, pericytes, and endothelial cells (red). Anti-LHβ (**a**), GH (**b**), TSHβ (**c**), PRL (**d**), and 17-39-ACTH (**e**) antibodies were used to detect hormone-producing cells, and anti-desmin antibody was used to detect pericytes (**f**). The anterior pituitary of transgenic S100β-GFP rats was used to identify folliculo-stellate (FS) cells (**g**). Laminin α1 was colocalized only with LHβ-positive cells (gonadotrophs). Bar=10 μm.

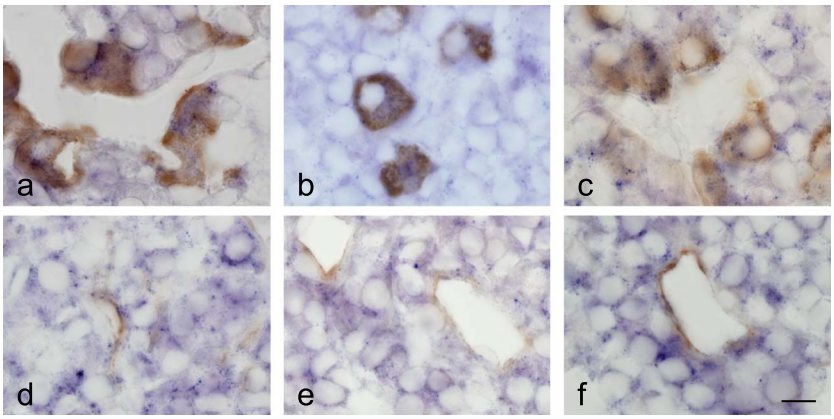

Fig. 5. Identification of laminin β and γ chains in rat anterior pituitary. *In situ* hybridization for laminin β1 (**a, d**), β2 (**b, e**), and γ1 (**c, f**), followed by immunohistochemistry for LHβ (**a–c**) or isolectin B4 (**d–f**). Cells expressing laminin β1, β2, and γ1 are shown in purple, and gonadotrophs and endothelial cells are in brown. All laminin β1, β2, and γ1 chains were expressed in gonadotrophs, while laminin β1 and γ1 chains were expressed in endothelial cells. Bar=10 μm.

isoforms present in rat anterior pituitary. We found that gonadotrophs produce two types of laminin isoforms (laminin 111 and 121), as do endothelial cells (laminin 411 and 311).

The present study using anti-laminin antibody (LSL-LB-1013) showed laminin deposition in the vascular basement membrane, interstitial space, and a subset of pituitary cells (Fig. 1). These results accord with previous *in vivo* and *in vitro* experiments, which confirmed laminin immunolocalization in both the vascular basement membrane and

some glandular cells of the anterior pituitary [9, 10, 22, 25]. Here, we successfully identified laminin α1, α3, α4, β1, β2, and γ1 in adult anterior pituitary (Fig. 2). Thus, the possible laminin isoforms expressed in this gland are laminin 111, 121, 311, 321, 411, and 421. We were also able to detect weak expression of laminin α2, α5, β3, γ2, and γ3 by RT-PCR, which suggests that laminin 211, 221, 212, 222, 213, 511, 521, 522, and 523 are also expressed in rat anterior pituitary, although expression levels might be very low.

The present study showed that the staining pattern for anti-laminin antibody (LSL-LB-1013) differed from that of anti-laminin α3 and α4 antibodies; the former stained the vascular basement membrane and the latter stained cytoplasm of endothelial cells (Figs. 1 and 3). This is probably due to the epitope of the antibodies: anti-laminin antibody (LSL-LB-1013) can recognize mature and assembled laminin isoforms [23], while the α chain-specific antibodies recognize only the unassembled laminin α chain, which is normally present in cytoplasm [14].

We also revealed that the only laminin-producing cells in the anterior pituitary were gonadotrophs and endothelial cells (Figs. 3–5). In contrast, Vila-Porcile *et al.* (1987) reported that laminin was localized in gonadotrophs, thyrotropes, and corticotropes and was scarce in lactotrophs [24]. Although the present results were not consistent with their findings, it should be noted that we examined expression levels of laminin α, β, and γ chains by using multiple approaches, including RT-PCR, immunohistochemistry and *in situ* hybridization, and laminin chain-specific gene probes and antibodies. We found that gonadotrophs produced laminin 111 and 121, while endothelial cells produced laminin 411 and a very limited amount of laminin 311 in the anterior pituitary.

The expression of laminin isoforms is cell- and tissue-specific, and these isoforms are believed to have varying functions in different tissues [8]. For instance, laminin 111, which was found to be expressed in gonadotrophs in the present study, induces cell differentiation in mammary gland cells [21] and supports neurite outgrowth in human mesenchymal stem cells [18]. Laminin 121 also affects neurite outgrowth [20], while laminin 311 transmits mechanosignals in lung epithelial cells [4]. It is widely believed that laminin 411, which was found to be produced by endothelial cells in the present study, mediates adhesion of platelets, leukocytes, and vascular endothelial cells [6]. Although further studies are required in order to reveal the specific function of each laminin isoform in the anterior pituitary, specific laminin isoforms may potentially have distinct roles in the anterior pituitary: laminin 111 might induce cell differentiation, for example, while laminin 411 may act specifically on vascular cell movement.

In conclusion, the present study is the first to identify the laminin isoforms and laminin-producing cells in the anterior pituitary. The present findings will improve understanding of the roles of laminin and the molecular basis of its involvement in regulating pituitary function.

V. Acknowledgments

We thank Prof. Kinji Inoue (Saitama University, Japan) for supplying the transgenic rats, David Kipler, ELS (Supernatant Communications) for revising the language of the manuscript, and Dr. Naoki Murayama (Murayama foundation) for generous support. This work was partly supported by a Grant-in-Aid for Scientific Research (C) (22590192) and by a Grant-in-Aid for Young Scientists (B) (23790233) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, by promotional funds for the Keirin Race of the Japan Keirin Association.

VI. Declaration of Interest

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

VII. Disclosure Statement

The authors have nothing to declare.

VIII. 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. R., 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. DOI: 10.1016/j.matbio.2005.05.006
2. 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.
3. Debbage, P. L., Griebel, J., Ried, M., Gneiting, T., DeVries, A. and Hutzler, P. (1998) Lectin intravital perfusion studies in tumor-bearing mice: Micrometer-resolution, wide-area mapping of microvascular labeling, distinguishing efficiently and inefficiently perfused microregions in the tumor. *J. Histochem. Cytochem.* 46; 627–639. DOI: 10.1177/002215549804600508
4. DeBiase, P. J., Lane, K., Budinger, S., Ridge, K., Wilson, M. and Jones, J. C. R. (2006) Laminin-311 (laminin-6) fiber assembly by type I-like alveolar cells. *J. Histochem. Cytochem.* 54; 665–672. DOI: 10.1369/jhc.5A6889.2006
5. Durbeej, M. (2010) Laminins. *Cell Tissue Res.* 339; 259–268. DOI: 10.1007/s00441-009-0838-2
6. Fujiwara, H., Kikkawa, Y., Sanzen, N. and Sekiguchi, K. (2001) Purification and characterization of human laminin-8. Laminin-8 stimulates cell adhesion and migration through α3β1 and α6β1 integrins. *J. Biol. Chem.* 276; 17550–17558. DOI: 10.1074/jbc.M010155200
7. Fujiwara, K., Jindatip, D., Kikuchi, M. and Yashiro, T. (2010) *In situ* hybridization reveals that type I and III collagens are produced by pericytes in the anterior pituitary gland of rats. *Cell Tissue Res.* 342; 491–495. DOI: 10.1007/s00441-010-1078-1
8. Hallman, R., Horn, N., Selg, M., Wendler, O., Pausch, F. and Sorokin, L. M. (2005) Expression and function of laminins in the embryonic and mature vasculature. *Physiol. Rev.* 85; 979–1000. DOI:10.1152/physrev.00014.2004
9. Hashimoto, H., Ishikawa, H. and Kusakabe, M. (1998) Three-

dimensional analysis of the developing pituitary gland in the mouse. *Dev. Dyn.* 212; 157–166.
10. Holck, S., Albrechtsen, R. and Wewer, U. M. (1987) Laminin in the anterior pituitary gland of the rat. Laminin in the gonadotrophic cell correlates with their functional state. *Lab. Invest.* 56; 481–488.
11. Horiguchi, K., Kouki, T., Fujiwara, K., Kikuchi, M. and Yashiro, T. (2011) The extracellular matrix component laminin promotes gap junction formation in the rat anterior pituitary gland. *J. Endocrinol.* 208; 225–232. DOI: 10.1677/JOE-10-0297
12. Itakura, E., Odaira, K., Yokoyama, K., Osuna, M., Hara, T. and Inoue, K. (2007) Generation of transgenic rats expressing green fluorescent protein in S-100-producing pituitary folliculo-stellate cells and brain astrocytes. *Endocrinology* 148; 1518–1523. DOI: 10.1210/en.2006-1390
13. Kikuchi, M., Sunaga, K., Yatabe, M., Takigami, S., Sakamoto, A. and Yashiro, T. (2005) An *in vivo* and *in vitro* immunohistochemical study of integrin alpha subunit localization in the rat anterior pituitary. *Acta Histochem. Cytochem.* 38; 381–386.
14. Kim, I., Ramakrishna, S., Gusarova, G. A., Yoder, H. M., Costa, R. H. and Kalinichenko, V. V. (2005) The forkhead Box M1 transcription factor is essential for embryonic development of pulmonary vasculature. *J. Biol. Chem.* 280; 22278–22286. DOI: 10.1074/jbc.M500936200
15. Lee, S. K., Park, S. C., Shrestha, P. and Mori, M. (1995) Expression of tenascin in fetal and adult salivary glands compared with the expression of type IV collagen and laminin-histomorphological roles. *Acta Histochem. Cytochem.* 28; 31–36.
16. Leong, A. S.-Y. and Leong, F. J. W.-M. (2002) Microwave-stimulated antigen retrieval—An update. *Acta Histochem. Cytochem.* 35; 367–374.
17. Li, S., Edgar, D., Fassler, R., Wadsworth, W. and Yurchenco, P. D. (2003) The role of laminin in embryonic cell polarization and tissue organization. *Dev. Cell* 4; 613–624.
18. Mruthyunjaya, S., Manchanda, R., Godbole, R., Pujari, R., Shiras, A. and Shastry, P. (2010) Laminin-1 induces neurite outgrowth in human mesenchymal stem cells in serum/differentiation factors-free conditions through activation of FAK–MEK/ERK signaling pathways. *Biochem. Biophys. Res. Commun.* 391; 43–48. DOI: 10.1016/j.bbrc.2009.10.158
19. Nunez, E. A., Pomeranz, H. D., Gershon, M. D. and Payette, R. F. (1990) Distribution of laminin in the murine pituitary. *Anat. Rec.* 226; 471–480.
20. Sasaki, T., Takagi, J., Giudici, C., Yamada, Y., Arikawa-Hirasawa, E., Deutzmann, R., Timpl, R., Sonnenberg, A., Bächinger, H. P. and Tonge, D. (2010) Laminin-121—recombinant expression and interactions with integrins. *Matrix Biol.* 29; 484–493.
21. Streuli, C. H., Schmidhauser, C., Bailey, N., Yurchenco, P., Skubitz, A. P., Roskelley, C. and Bissel, M. J. (1995) Laminin mediates tissue-specific gene expression in mammary epithelia. *J. Cell Biol.* 129; 591–603.
22. 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* 21; 57.
23. Tsuji, N., Kita, K., Ozaki, K., Narama, I. and Matsuura, T. (2011) Organogenesis of mild ocular coloboma in FLS mice: failure of basement membrane disintegration at optic fissure margins. *Exp. Eye Res.* 94; 174–178. DOI: 10.1016/j.exer.2011.12.004
24. Vila-Porcile, E., Picart, R., Tixier-Vidal, A. and Tougard, C. (1987) Cellular and subcellular distribution of laminin in adult rat anterior pituitary. *J. Histochem. Cytochem.* 35; 287–299.
25. Yurchenco, P. D. (1990) Assembly of basement membranes. *Ann. N. Y. Acad. Sci.* 580; 195–213. DOI: 10.1111/j.1749-6632.1990.tb17929.x