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学 位 論 文 名	ラット下垂体前葉における基底膜主要成分であるラミニンの アイソフォームに関する研究
論 文 審 査 委 員	(委員長) 教 授 田 中 亨 (委 員) 教 授 渡 辺 英 寿 教 授 石 川 三 衛

## 論文内容の要旨

### 1 研究目的

Laminin is a major component of basement membrane. It consists of a single  $\alpha$ ,  $\beta$ , and  $\gamma$  chains. So far, there are 5 $\alpha$ , 3 $\beta$ , and 3 $\gamma$  chains identified. By the combination of 3 chains, they can assemble to form 19 laminin isoforms (eg, laminin 121 is composed of  $\alpha$ 1,  $\alpha$ 2, and  $\alpha$ 1). Laminin isoforms are required for basement membrane assembly and regulate cellular function via interactions with cell surface receptors.

Recently, our research group showed that laminin interacts with anterior pituitary cells and promotes cell proliferation, migration, and regulates gene transcription. Since cell proliferation, migration, and differentiation occur during pituitary development and tumorigenesis, it is essential to consider whether certain laminin isoforms contribute to these events. However, no report has identified the laminin isoforms expressed during different stages of anterior pituitary development and tumorigenesis. The purpose of this study is to localize the laminin isoforms and laminin-producing cells in rat anterior pituitary adult and development and tumorigenesis.

### 2 研究方法

#### Experiment I

The aim of this experiment is to identify laminin isoforms and laminin producing cells in the anterior pituitary gland of adult rats. To localize laminin in the gland, immunohistochemistry using anti-laminin antibody was first performed. After RT-PCR using gene-specific primers to determine laminin chains expressed in the gland,  $\alpha$  chain-expressing cells were characterized by immunohistochemistry using  $\alpha$  chain-specific antibodies. Regarding the localization and expression of  $\beta$  and  $\gamma$  chain, *in situ* hybridization followed by

immunohistochemistry was performed.

#### Experiment II

The aim of this experiment is to comprehend identification of laminin chains during development of rat pituitary gland. To this end, *in situ* hybridization was used to determine the laminin  $\alpha$ ,  $\beta$ , and  $\gamma$  chains expressed in prenatal stages (Embryonic day 12.5, 15.5, and 19.5) and postnatal stages (Postnatal day 5, 10, and 30) using chain-specific probes.

#### Experiment III

The aim of this experiment is to identify laminin  $\alpha$  chains in tumorigenesis of prolactinoma. As a prolactinoma model animal, diethylstilbestrol (DES)-treated rats were used. Laminin  $\alpha$  chain expression during tumorigenesis of prolactinoma (0-, 4-, 8-, and 12-week DES treatment) was determined by *in situ* hybridization and immunohistochemistry.

### 3 研究成果

#### Experiment I

This experiment identified the expression of laminin isoforms and laminin producing cells in the adult rat anterior pituitary by RT-PCR, immunohistochemistry, and *in situ* hybridization. RT-PCR showed that laminin  $\alpha 1$ ,  $\alpha 3$ , and  $\alpha 4$  genes were expressed in anterior pituitary. By immunohistochemistry, laminin  $\alpha 1$  was detected in gonadotrophs, while laminin  $\alpha 4$  was observed in almost all vascular endothelial cells. Laminin  $\alpha 3$  was seen in a subset of vascular endothelial cells. The author further performed *in situ* hybridization to localize  $\beta$  and  $\gamma$  chains in these cells. The result showed that laminin  $\beta 1$ ,  $\beta 2$ , and  $\gamma 1$  were expressed in gonadotrophs and laminin  $\beta 1$  and  $\gamma 1$  in endothelial cells.

#### Experiment II

This experiment investigated the expression and localization of all laminin chains in pre- and postnatal stages of rat pituitary gland by *in situ* hybridization. The  $\alpha 5$  chain was expressed during early pituitary development (E12.5–15.5). Expression of  $\alpha 1$  and  $\alpha 4$  chains was noted in vasculature cells at E19.5, but later diminished. The  $\alpha 1$  chain was re-expressed in parenchymal cells of anterior lobe from P10, while the  $\alpha 4$  chain was present in vasculature cells from P30. The  $\alpha 2$  and  $\alpha 3$  chains were transiently expressed in vasculature cells and anterior lobe, respectively, only at P30. Widespread distribution of  $\beta$  and  $\gamma$  chains was also observed during gland development.

#### Experiment III

This experiment examined  $\alpha$  chain expression during tumorigenesis of prolactinoma (0- to 12-week DES treatment) by *in situ* hybridization and

immunohistochemistry. The mRNA of  $\alpha 1$ ,  $\alpha 3$ , and  $\alpha 4$  chains was detected in control and 4-week DES, and their expressions were decreased in 8-week DES and prolactinoma. Immunohistochemistry showed that the  $\alpha 1$  chain was localized in some anterior pituitary cells of control and 4-week DES, and in endothelial cells of 8-week DES. The  $\alpha 3$  and  $\alpha 4$  chains were expressed in endothelial cells, and their immunoreactivities and number of immunopositive cells were decreased during DES treatment.

#### 4 考察

##### Experiment I

The author identified that  $\alpha 1$ ,  $\alpha 3$ ,  $\alpha 4$ ,  $\beta 1$ ,  $\beta 2$ , and  $\gamma 1$  chains were expressed in adult anterior pituitary. The possible combinations of laminin isoforms expressed in this gland were laminin 111, 121, 311, 321, 411, and 421. The author also revealed that gonadotrophs and endothelial cells produced laminin in rat anterior pituitary. Intriguingly, the isoforms were different by cell types: Laminin111 and 121 in gonadotrophs and laminin 411 and 311 in endothelial cells.

##### Experiment II

The author revealed that laminin isoforms containing  $\alpha 5$  are the first isoforms expressed at E12.5 and are expressed before differentiation of hormone-producing cells in rat anterior pituitary (at E13). The isoforms might support initial invagination of the oral ectoderm and subsequent formation of the pituitary anlage. Laminin containing  $\alpha 1$  were first expressed in vasculature cells of the anterior lobe at E19.5, and disappeared thereafter and re-expressed in gonadotrophs. Gonadotrophs maturation is known to occur after the first postnatal week, suggesting that maturing/matured gonadotrophs produce laminin containing  $\alpha 1$ . Expression of the  $\alpha 4$  chain was noted in vasculature cells at E19.5 and diminished at P5-P10 and was re-expressed in the vasculature at P30-P60. Since temporal alteration of  $\alpha 4$  chain expression has not been reported during vascular development in other tissues, this phenomenon might be associated with distinct vascular development and maturation in the pituitary gland.

##### Experiment III

Previous studies using DES-treated LEXF RI rats have suggested that prolactinoma was induced by 12-week DES treatment. By immunohistochemistry, the  $\alpha 1$  chain was stained in gonadotrophs in control and 4-week DES treatment, but not in endothelial cells. The  $\alpha 1$  chain-immunopositive LH cells disappeared after 8-week DES treatment. It is suggested that  $\alpha 1$  chain expression in LH cells may respond to DES. Interestingly, in 8-week DES treatment, the  $\alpha 1$  chain was expressed in a few of endothelial cells, but not in gonadotrophs. It is suggested that temporally-expressed  $\alpha 1$  chain in 8-week DES treatment might be associated

with neovascularization in prolactinoma. The  $\alpha 4$  chain immunoreactivity and the number of  $\alpha 4$  chain-immunopositive cells were decreased after 8-week DES treatment. Since previous study showed that deletion of the  $\alpha 4$  chain leads to impaired capillaries maturation, our data suggest that  $\alpha 4$  chain is important in maturation of vascular basement membrane.

## 5 結論

A series of study identified laminin chains and laminin-producing cells in adult rat anterior pituitary, development of pituitary, and tumorigenesis, and found that the expression pattern of laminin isoforms was dependent on the context of anterior pituitary development and tumorigenesis. The author believes that the results of this study will shed the light on the future research in anterior pituitary function, development, and disease.

## 論文審査の結果の要旨

本研究は下垂体におけるラミニン鎖の発現変化を主に免疫組織化学と *in situ hybridization* により解析したものである。2重染色法なども用いて鮮明な画像を提示しており、形態学的な手技のレベルは高い。申請者は、まず *adult* の下垂体で解析を行い、細かな血管網においてもラミニンが分布していることを示し、基底膜構造が光顕レベルでは明確ではない下垂体において、分子レベルでは明確に構造が形成されていることを示した。さらに、申請者は、血管内皮細胞以外に LH 発現細胞でラミニン産生が生じていることも同時に明らかにした。下垂体においてラミニン発現パターンについては確立した見解は得られていなかったが、網羅的な本研究により、ほぼ確定したといえる。申請者は、さらに、下垂体の発生や発達段階でのラミニン発現についても解析を行い、アイソフォームが発生、発達により変化することを明確に示した。下垂体は微小な器官であり、どの分子でも発生や発達段階で丹念に検討するには困難な組織であるが、非常に丁寧に検討が成されており、レベルの高い研究になっている。最後に、下垂体腫瘍モデルでのラミニン発現についての検討も示された。この研究では、プロラクチノーマしか扱っておらず、若干不十分な内容ではあるが、今後、ヒト腫瘍などでの応用が期待される内容とはなっている。

委員のなかからは、ラミニンの発現変化と細胞機能との関係が十分に示されていない点が指摘されたが、申請者もその点に関しては今後の検討課題と十分に認識しており、大きな欠点とは言えない。

以上により、全員一致で合格と判定した。

## 最終試験の結果の要旨

申請者は、基底膜やラミニンの基本構造を十分に理解し、シェーマや電顕写真などを用いて非常にわかりやすい説明がなされた。次に、ラミニンがインテグリンと作用して機能を示すなどが説明され、機能的な知識も十分に持ち合わせている。本研究の基礎となる下垂体の基本的な構造や発達・分化について、難解な発生段階の構造変化をわかりやすく提示し、形態学の素養の深さを感じさせた。さらに、免疫染色や *in situ hybridization* の判定における陰性コントロールの重要性や非特異的な陽性反応に対する注意点なども良く理解しており、初心者が陥る様な偏った解釈もない。また、形態学的な研究の限界も良く理解しており、今回の研究内容から言える点、言えない点をきっちりとわきまえている。今後の研究の課題や方向性に対しても非常に明確に認識しており、博士号にふさわしいレベルであることは明らかである。

以上から、全員一致で最終試験合格と判定した。