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Expression of Transforming Growth Factor-β Superfamily Receptors in Developing Rat Eyes

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Purpose: To ascertain the role played by the transforming growth factor (TGF)-β superfamily in retinal development by determining the changes in the expression patterns of their receptors during development of the normal rat retina.

Methods: The expression of type I and type II receptors of the TGF-β superfamily was observed at the protein level in rat eyes at embryonic age 17 days (E17), at birth (P0), at postnatal days 3, 6, 9, and at 11 weeks (P3, P6, P9, and adult, respectively).

Results: Activin type I receptor and BMP type IB receptor were first detected in P6 and P3 retinas, respectively, at the protein level, and activin type II receptor was first detected in the P0 retina. The other receptors (TGF-β type I and II receptors, activin type IB receptor, BMP types IA and II receptors) were detected at E17. The period from P0 to P9 corresponded to the period of dynamic changes in the rat retinal development.

Conclusion: The results suggest that the expression of TGF-β superfamily is regulated along with retinal development and may be related to retinal development. **Jpn J Ophthalmol 1999;43:290–294** © 1999 Japanese Ophthalmological Society

Key Words: Activins, bone morphogenetic proteins (BMP), rat retinal development, transforming growth factor β, type I and type II receptors.

Introduction

The transforming growth factor-β (TGF-β) superfamily is composed of several families of multifunctional proteins that are structurally related and include the TGF-β family, the activin inhibin family, and the bone morphogenetic protein (BMP) family.[1,2] Members of the TGF-β superfamily have been shown to regulate cell proliferation, differentiation, adhesion, migration, and production of extracellular matrix components. Some of these proteins, including TGF-βl, TGF-β2, TGF-β3, activin A, inhibin, and OP-1 (BMP-7), are expressed in ocular tissues.[3–13] The absence of OP-1 or TGF-β2 has been found to affect the development of mouse eyes.[12,14–16] These findings suggest that the TGF-β superfamily members regulate cell function during normal development and maintain the homeostasis after birth.

The TGF-β superfamily members transduce their signals by binding to specific type I and type II receptors that have serine/threonine kinase activity. Both types of receptors are indispensable for signal transduction.[1,2,17,18] Seven type I receptors, denoted as activin receptor-like kinase-1 (ALK-1) through ALK-7, have been cloned.[18–23] ALK-5 was determined to be the TGF-β type I receptor (TβR-I),[2,19] whereas ALK-2 and ALK-4 are activin receptors ActR-I and ActR-IB, respectively. ALK-3 and ALK-6 are functional BMP receptors, BMPR-IA and BMPR-IB, respectively.[2,18,20,21] ActR-I also functions as a BMP type I receptor.[l8,24]

The type II receptor family has been shown to include the TGF-β type II receptor (TβR-II), two activin receptors (ActR-II and ActR-IIB) and a BMP receptor (BMPR-II). ActR-II and ActR-IIB also function as BMP type II receptors.[2,18,24,25]

To determine the functions or target cells of the TGF-β superfamily members during retinal development, it is necessary to determine the expression patterns of type I and type II receptors during the development of the normal retina.[26–28] We therefore

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followed the changes of the expression patterns of five type I receptors and three type II receptors during the development of normal rat retinas at the protein level.

Materials and Methods

Animals

Lewis rats were used in this study. The eyes of the Lewis rats were enucleated at embryonic age 17 days (E17) and on 0, 3, 6, and 9 days, and 11 weeks postnatally (designated as P0, P3, P6, P9, and adult, respectively). Three rats at each age were sacrificed for histological observation. The rats were treated according to "Principles of Laboratory Animal Care," NIH publication, No. 86-23, revised 1985.

Histological Observations

Enucleated eyes were frozen in Tissue-Tek OCT compound® (Miles Laboratories, Naperville, IL, USA). Tissue sections (6-μm thick) were mounted on poly-L-lysine–coated slides. The frozen sections were fixed for 5 minutes in ice-cold 4% paraformaldehyde in 0.1 mmol/L phosphate buffer (pH 7.4), and then rinsed in phosphate-buffered saline (PBS, pH 7.4).

For immunohistochemical observation, the sections were treated for 15 minutes with 3% hydrogen peroxide in PBS to block endogenous peroxidase activity. After rinsing in PBS, the slides were incubated with nonimmune goat serum for 20 minutes at room temperature (RT) to block nonspecific antibody binding. After another rinse in PBS, the sections were incubated overnight at 40°C with a primary antibody. Using a Histofine SAB-PO kit® (Nichirei Corporation, Tokyo), the sections were incubated for 15 minutes at RT with biotinylated anti-rabbit goat serum, rinsed in PBS, and then incubated for 10 minutes with streptoavidin–biotin–peroxidase complex at RT. The final reaction product was visualized with 3,3'-diaminobenzidine tetrahydrochloride (DAB), after which the sections were counterstained with hematoxylin. The antibodies were used at a final concentration of 3 μg/mL. For negative control, the sections were incubated with PBS, nonimmune IgG, or primary antiserum, and then absorbed for 60 minutes at RT with an excess of the peptide used to generate the corresponding antiserum.

Antibodies

Dr. Kohei Miyazono and Dr. Mitsuyasu Kato (The Cancer Institute, Tokyo) supplied the primary antibodies. These antibodies were: polyclonal rabbit antibodies directed against TGF-β type I receptor (anti–TβR-I), activin type I receptor (anti–ActR-I), activin type IB receptor (anti–ActR-IB), BMP type IA receptor (anti–BMPR-IA), BMP type IB receptor (anti–BMPR-IB), anti-activin type II receptor (anti–ActR-II), TGF-β type II receptor (anti–TβR-II), and BMP type II receptors (anti–BMPR-II). Two alternative spliced forms of BMPR-II have been reported.[24,29] Two antibodies to BMPR-II were generated against peptides corresponding to amino acid residues 185–202 and 534–556, and designated as SMN and NRR, respectively. SMN and NRR were named according to the first letter of 3 amino acid residues of the polypeptide sequences: SMN = Ser-Met-Asn, and NRR = Asn-Arg-Arg. The truncated form of BMPR-II was detected only by SMN and not by NRR, whereas the longer form was detected by both SMN and NRR.[24,29] The specificity of each antibody was characterized biochemically and they were reported not to cross-react with each other.[19,21,24] The antibody to rhodopsin was purchased from Cosmo Bio Co. (Tokyo).

Results

Morphological Development of the Rat Retina

In embryonic (El7) and early postnatal (P0, P3) rats, a single nuclear layer was observed in the retina. The separation of the inner and outer nuclear layers proceeded from the central retina toward the peripheral retina and was complete by about 6 days of age.[26–28] Rhodopsin, a marker of the outer segment of the photoreceptor, was first weakly detected immunohistochemically in P9 retinas (data not shown).

Expression of the Receptors

The expression of TβR-II and BMPR-II was observed immunohistochemically in E17 to P3 retinas to be positive in the ganglion cell layer and in the single nuclear layer (Figure 1); it is also positive in the ganglion cell layer, the inner and outer nuclear layers, the inner segments of the photoreceptor layer, the retinal pigment epithelial layer, and the choriocapillaris layer in P6 to adult retinas (Figure 1). ActR-II expression was detected in P0 retinas. These observations are summarized in Table 1.

The expression pattern of type II receptors showed different patterns in the outer segments of photoreceptor layer. Outer segments of the photoreceptors were stained by SMN but not by NRR indicating that only the truncated BMPR-II was ex-

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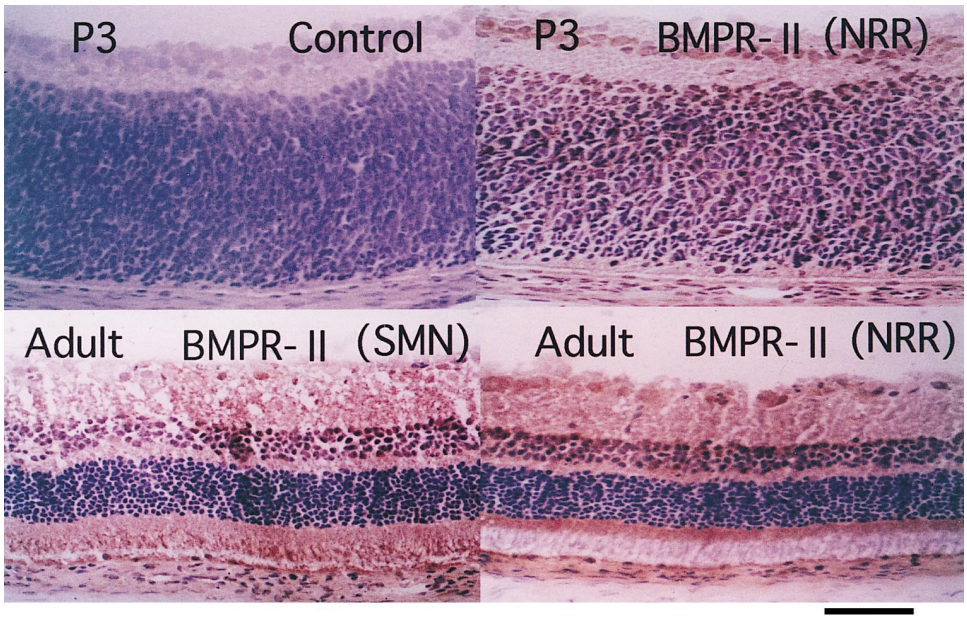

Figure 1. Immunohistochemical observations on expression of BMPR-II in P3 and adult retinas. BMPR-II, longer version (detected by NRR antibody), is observed in ganglion cells and nuclear cell layer before separation in P3 retina (top right). Top left: Negative control of P3 retina using nonimmune IgG in place of NRR . The two antibodies to BMPR-II showed different patterns of expression in outer segments of the photoreceptors of adult retinas (bottom right and left). Outer segments are stained by SMN and not by NRR, which shows that truncated BMPR-II is expressed in outer segments. (Original magnification: × 150.) Bar = 100 μm.

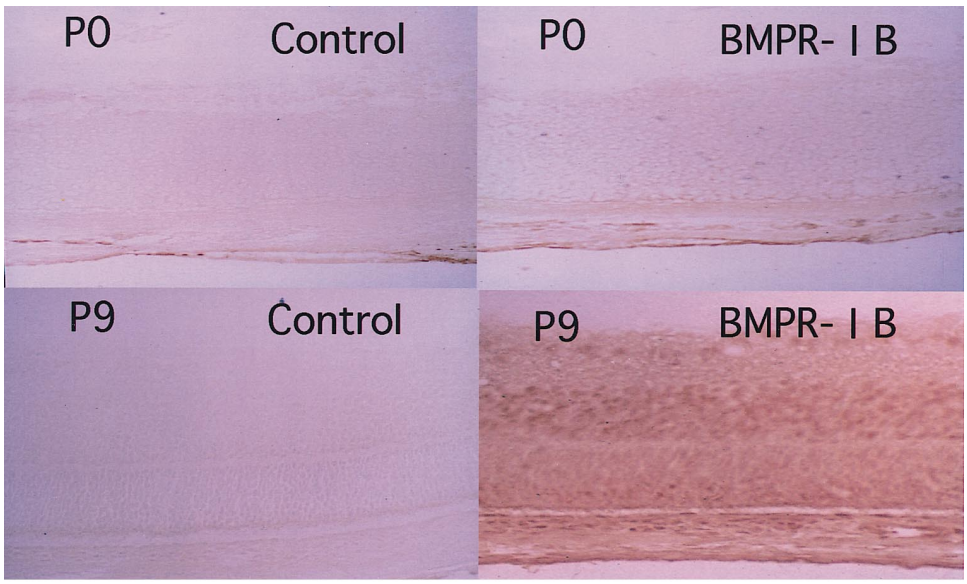

Figure 2. Immunohistochemical observations on expression of BMPR-IB in P0 and P9 retinas. BMPR-IB is not observed in P0 retina but is observed in ganglion cells, inner and outer nuclear layers, and choroidal tissue of P9 retina. Top left: Negative control of P0 retina using nonimmune IgG as primary antibody. Top right: Staining with antibody to BMPR-IB in P0 retina. Positive staining is not detected. Bottom left: Negative control of P9 retina using nonimmune IgG as primary antibody. Bottom right: Staining with antibody to BMPR-IB in P9 retina. Positive staining is in the ganglion cell layer, inner and outer nuclear layers, and choroidal tissue. (Original magnification: × 150.) Bar = 100 μm.

Table 1. Expression of Type I and Type II Receptors in Developing Rat Retina

						Adult	
Type I	E17	P0	P3	P6	P9	Retina*	OS†
TβR-I	±	+	+	+	+	+	−
ActR-I	−	−	−	±	+	+	−
ActR-IB	+	+	+	+	+	+	−
BMPR-IA	+	+	+	+	+	+	−
BMPR-IB	−	−	±	±	+	+	−

						Adult	
Type II	E17	P0	P3	P6	P9	Retina*	OS†
TβR-II	+	+	+	+	+	+	+
ActR-II	−	+	+	+	+	+	−
BMPR-II (NRR)	±	±	+	+	+	+	−
BMPR-II (SMN)	±	+	+	+	+	+	+

*Retina excluding outer segment of photoreceptor.

†Outer segment of photoreceptor. NRR: longer version of BMPR-II detected by antibody NRR. SMN: truncated BMPR-II detected by antibody SMN.

+: Positive; ±: weakly positive; −: negative.

pressed in the outer segments (Figure 1). TβR-II was also expressed in the outer segments, and ActR-II was not expressed in the outer segments (Table 1).

ActR-I and BMPR-IB were first detected in the P6 and P3 retinas, respectively (Figure 2). Positive staining was detected in the ganglion cell layer, inner nuclear layer, outer nuclear layer, retinal pigment epithelial layer, and choriocapillaris layer (Figure 2). All of the other receptors (TPR-I, ActR-IB, BMPR-IA) were expressed as early as the E17 retina (positive for ganglion cell layer, and unseparated single nuclear layer). Type I receptors were not expressed in the outer segments of adult rat retina that were similar to BMPR-II (NRR) (see Figure 1). These results are summarized in Table 1.

Discussion

The time of receptor expression during retinal development was different for the type I and type II receptors (Table 1). ActR-I and BMPR-IB were first detected in the P6 and P3 retinas, respectively, whereas ActR-II was first detected at P0 (Table 1). All other receptors examined (TβR-I, TβR-II, ActR-IB, BMPR-IA, BMPR-II) were expressed as early as the E17 stage (Table 1). ActR-I first appeared at the P6 stage, whereas ActR-II was already expressed at the P0 stage. This suggests that the effects mediated by activin and/or BMP through ActR-I and ActR-II may occur 6 days after birth in the retina (Table 1).[26,28]

The appearance times were different for ActR-I and ActR-IB, which are receptors for activin. This supports the idea that the functions of these two receptors are different.[22] Both TGF-β type I and type II receptors were expressed at E17 when TGF-β exerts specific effects on the developing retina (Table 1).[17–19] We also observed differences in the time of expression for the three types of BMP type I receptors (ActR-I, BMPR-IA, BMPR-IB) (Table 1). This suggests that the effects mediated by BMP through BMPR-IA and BMPR-II occur first, then those through BMPR-IB and BMPR-II, and finally those through ActR-I and BMPR-I.[12,18,20,24,29] The responsible ligands in the activin and/or the BMP family for these pairs of receptors remain to be investigated in the future. Activin A and OP-l (BMP-7) are considered as the possible ligands.[6,12,15]

The 3- to 9-day period after birth, when the structure of the retina changes drastically, is a very important period for the development and differentiation of the rat retina.[26–28] For example, the inner and outer nuclear layers separate at 3–6 days after birth, the photoreceptor inner segment appears around 6 days, and rhodopsin begins to be expressed around 9 days. Because activin and BMP were found to begin to exert their effects at different times along with the morphological changes of the retina as described above, these findings suggest that the TGF-β superfamily members exert important effects at different times during the development of retina. The responsible ligands in the activin and/or the BMP family for these pairs of receptors remain to be investigated in the future.

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