Bone Morphogenetic Proteins-2 and -4: Negative Growth Regulators in Adult Retinal Pigmented Epithelium

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PURPOSE. To determine the relative level and localization of bone morphogenetic protein (BMP)-4 mRNA in the retina and retinal pigmented epithelium (RPE) under normal and pathologic conditions, to seek clues regarding possible functions.

METHODS. Clones isolated from an RPE cDNA library were sequenced and used as probes for northern blot analysis. Expression in the retina and RPE was investigated in mouse models using reverse transcription–polymerase chain reaction (RT-PCR) and in situ hybridization. The effect of recombinant proteins on RPE proliferation was investigated by thymidine incorporation.

RESULTS. Bovine clones with high homology to BMP-2 and BMP-4 were isolated from a subtracted RPE cDNA library. Northern blot analysis using the clones as probes demonstrated abundant and differential expression in adult bovine RPE, but with RT-PCR and in situ hybridization, expression was also demonstrated in mouse retinal neurons. In mice with oxygen-induced ischemic retinopathy there was a striking decrease in BMP-4 mRNA in the retina within 6 hours of the onset of hypoxia that was maintained for at least 5 days. In mice with inherited photoreceptor degeneration, there was a dramatic decrease in BMP-4 mRNA in retina and RPE during and after the degeneration. mRNA for the type II BMP receptor was observed in freshly isolated and cultured RPE cells, isolated retina, and freshly isolated bovine aortic endothelial cells. Thymidine incorporation in early-passage RPE cells showed a 14-fold stimulation above control with 5% serum that was decreased to 322%, 393%, and 313% in the presence of BMP-2 (10 ng/ml), BMP-4 (10 ng/ml), and transforming growth factor (TGF)-β1 (2 ng/ml), respectively.

CONCLUSIONS. BMP-2 and BMP-4 may serve as negative growth regulators in the retina and RPE that are downregulated by injury, to allow tissue repair. Modulation of expression of the BMPs may provide a means to control the exaggerated wound repair that occurs in proliferative retinopathies.

Invest Ophthalmol Vis Sci. 2000;41:592–600

Bone morphogenetic proteins (BMPs) were found to be components of bone extracts that induce ectopic bone formation when injected into animals.1 At least 10 BMPs have been identified. BMP-1 is a procollagen C proteinase2 and the other BMPs are members of the transforming growth factor (TGF)-β superfamily. BMP-2 and BMP-4 are more closely related to each other and to the gene product of the Drosophila decapentaplegic (dpp) gene, than other BMPs and constitute the DPP subfamily.3

BMP-2 and BMP-4 play an important role in embryonic development. BMP-4 induces formation of the ventral ectoderm and mesoderm and thereby helps to establish the dorsal–ventral axis in early Xenopus embryos.4,5 It has a similar critical function in vertebrates; mice with targeted disruption of Bmp-4 die early in embryonic development and show little or no mesodermal differentiation.6 BMP-2 and -4 also participate in later developmental processes, including outgrowth and patterning of facial primordia,7 patterning of limb buds in which BMP signaling stimulates apoptosis leading to regression of tissue between digits,8,9 and induction of cardiac myogenesis.10

Members of the TGF-β superfamily often have multiple soluble and cell surface binding proteins that mediate and/or modulate their signaling. As is the case with other family members, there is a system of receptor cooperativeness between type I and type II receptors, with the type II BMP receptor playing a primary role.11 It is a serine-threonine kinase...
that has substantial homology to the type II receptors of other family members.

To our knowledge, a function for BMPs in adults has not been identified. Therefore, it was surprising that, while using techniques designed to isolate genes that are differentially expressed in the adult retinal pigmented epithelium (RPE) and retina, 12-14 we isolated bovine clones that show high homology to human BMP-2 and BMP-4. In this study, in an effort to gain insight into the role of BMPs in adult eyes, we examined the expression pattern of BMP-2 and BMP-4 in the retina and RPE, the modulation of BMP-4 mRNA levels in animal models, and the effect of recombinant human BMPs on cultured human RPE cells.

**METHODS**

**Isolation of Bovine Clones Homologous to BMP-2 and -4 from an RPE cDNA Library**

A cDNA library was constructed (in AUni-ZAP XR; Stratagene, La Jolla, CA), by using cDNA generated from bovine RPE RNA. Two approaches were used to identify genes that are differentially expressed in the RPE. The first was differential hybridization. Approximately 5000 plaques were plated on Luria-Bertani (LB) agar plates and transferred to duplicate nitrocellulose membranes. One membrane was hybridized with 32P-labeled cDNA probe made from bovine RPE RNA, and the other was hybridized with 32P-labeled cDNA probe made from bovine liver RNA. Several clones that gave a strong signal with RPE probe but little or no signal with liver probe were sequenced.

The second approach was subtractive hybridization. 12-14 The bovine RPE library was excised in vivo, made single stranded, and hybridized in several rounds with an excess of biotinylated heart and liver RNA. The resultant RNA-DNA hybrids and unhybridized RNA were removed by phenol extraction after the addition of streptavidin, and the remaining unhybridized plasmid DNA was electroporated into MC1061 cells. Approximately 1000 clones from the subtracted library were partially sequenced.

**Northern Blot Analysis**

RNA was isolated from several bovine tissues, human retina, human RPE, cultured human RPE, and murine retina. Ten micrograms of total RNA was fractionated on formaldehyde-containing 1.2% agarose gels and transferred to Nytran paper (Cuno, Meriden, CT). Bovine or murine clones homologous to BMP-2, BMP-4, or TGF-β were labeled with 32P using a random priming kit according to the manufacturer’s instructions (Stratagene). Hybridizations were performed at 42°C for 20 hours in 50% formamide, 5× SSPE, 5× Denhardt’s solution, 0.1% sodium dodecyl sulfate (SDS), and 150 μg/ml denatured salmon sperm DNA. Two 15-minute washes were performed at 42°C in 2× SSPE-0.1% SDS, followed by one rinse at 42°C in 0.1× SSPE with 0.1% SDS and a final rinse at 60°C in 0.1× SSPE-0.1% SDS. Washed blots were exposed to xray film (XAR; Eastman Kodak, Rochester, NY) with an intensifier screen at −80°C. Exposure times were varied for optimal visualization of results. Blots were then stripped and rehybridized with a probe for 18S ribosomal RNA to control for possible differences in RNA loading.

**Reverse Transcription–Polymerase Chain Reaction**

RNA was isolated from developing mouse brain or cultured human RPE. Reverse transcription was performed with 1 μg RNA, reverse transcriptase (Gibco, Gaithersburg, MD) and 20 μM gene-specific 3’ primer. Aliquots of the cDNAs were used for PCR amplification using oligonucleotide primers corresponding to nucleotides 257 through 276 (forward, 5’-ACTGCGCGACGGTTCTGAG-3’) and 742 through 725 (reverse, 5’-TCCTCCAGATGTTCTTCTGAG-3’) of murine BMP-4, nucleotides 531 through 552 (forward, 5’-ACAGAATGGTACCGAGGACGGC-3’) and 1187 through 1166 (reverse, 5’-CTCTCTTCTAGACTTCTGGTGC-3’) of murine type II BMP receptor, and to nucleotides 1497 through 1523 (forward, 5’-GGAATTGACGATCTACTAGCCACCGAG-3’) and 1882 through 1910 (reverse, 5’-TTCCGGCGCGACGAGCTTAATGAAT-3’) of murine TGF-β2. There is high homology among the mouse, human, and bovine sequences for these regions of the genes, and the same primers were used to amplify appropriate products from human and bovine reverse transcription–polymerase chain reaction (RT-PCR). However, no product could be obtained for BMP-4 from RNA isolated from bovine aortic endothelial cells. Therefore, additional primers were designed from bovine BMP-4 sequence (forward, 5’-CATCTGGAGAGATCCTCCAGC-3’) and reverse, 5’-GATCGCTGAATCCTGACAT-3’). Thirty-three cycles of amplification were performed, and products were subcloned into pBluescript (Stratagene) or pNoTa/T7 (5’-3’, Boulder, CO) and sequenced to confirm their identities. The same primers were used for semiquantitative RT-PCR of BMP mRNA levels in retina in combination with primers for 16S ribosomal RNA, as previously described. 15

**Obtaining Infant and Adult Mouse Retinas for Expression Studies**

Mice were used in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. C57BL/6J mice were killed at several time points (postnatal days [P]0, P3, P5, P10, and P17 and adult) spanning the period of retinal vascular development, and eyes were frozen in optimal cutting temperature embedding compound (OCT; Miles Diagnostics, Elkhart, IN) for frozen sections. Eyes from BALB/c albino mice, C3H rd mice, FVB albino rd mice, and wild-type C3H and FVB mice were also obtained.

**Murine Model of Ischemic Retinopathy**

Ischemic retinopathy was produced in wild-type C57BL/6J mice as described by Smith et al. 16 P7 mice and their mothers were placed in an airight incubator and exposed to an atmosphere of 75% ± 5% oxygen for 5 days. Incubator temperature was maintained at 23 ± 2°C, and oxygen concentration was measured and adjusted every 12 hours with an oxygen analyzer. After 5 days, the mice were removed from the incubator. The mice were killed immediately after removal from the hyperoxic environment (0 hours), and after 6, 12, and 24 hours and 5 days in room air, and control animals were killed at P12 and P17. Eyes were rapidly removed, embedded in OCT compound, rapidly frozen in a bath of 2-methylbutane and dry ice, and stored at −80°C until they were sectioned.

**In Situ Hybridization**

Sense and antisense digoxigenin-labeled riboprobes for BMP-4 were synthesized by in vitro transcription using the bovine clone.
as a template, as previously described. Cryosections 12-μm thick were fixed in 4% paraformaldehyde, treated with pronase E, and postfixed with 4% paraformaldehyde. In situ hybridization was performed using sense and antisense probes in a concentration of 200 to 500 ng/ml at 55°C for 18 hours. Posthybridization washes were performed at 55°C followed by RNase treatment. Sections were blocked with 40% heat-inactivated lamb serum at 4°C for 1 hour and treated with alkaline phosphatase–conjugated anti-digoxigenin antibody overnight, followed by postantibody washes. An alkaline phosphatase–mediated color reaction was performed. Slides were examined by microscope (Axioskop; Carl Zeiss, Thornwood, NY), and images were digitized using a color video camera (3 CCD; IK-TU40A; Toshiba, Tokyo, Japan) and a frame grabber. Image analysis software (Image-Pro Plus; Media Cybernetics, Silver Spring, MD) was used for densitometry on P17 ischemic and control retinas.

Cell Culture

Human RPE cells were cultured from eyes obtained from the Old Dominion Eye Bank (Richmond, VA) using a procedure described previously. RPE cells were grown in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Upstate Biotechnologies, Lake Placid, NY). The ARPE-19 RPE cell line that has differentiated characteristics, including spontaneous expression of CRALBP and formation of a polarized monolayer with high transepithelial resistance when grown on porous filters was provided by Leonard Hjelmeland (University of California, Davis). RPE cells were grown in DMEM supplemented with 5% or 10% FBS. For some experiments, cells were maintained in serum-free M199 with 0.1% bovine serum albumin (BSA). Cultures were demonstrated to be pure populations of RPE cells by immunocytochemical staining for cytokeratins.

Aortic endothelial cells were isolated from bovine aortas by mild digestion with 0.04% trypsin and 0.05% EDTA for 2 minutes followed by gentle scraping of the intima. Total RNA was isolated (Purescript RNA Isolation Kit; Gentra Systems; Minneapolis, MN) according to the manufacturer’s instructions.

Tritiated Thymidine Incorporation

RPE cells at passage three were grown to confluence in 24-well plates, placed in serum-free medium for 24 hours, and incubated in medium containing recombinant human BMP-2 or -4 (Genetics Institute, Cambridge, MA) or recombinant human TGF-β3 (R&D Systems, Minneapolis, MN) or medium alone in the presence or absence of 2.5% or 5% serum. After 16 hours, the cells were pulsed with 2 μCi/ml [3H]thymidine (specific activity 6.7 Ci/mmol; NEN, Boston, MA) for 2 hours. The cells were washed three times with PBS and five times with ice-cold 5% trichloroacetic acid. One milliliter of 0.1 M NaOH containing 0.1% SDS was added to each well, and after 1 hour a 100-μl aliquot was counted in a Wallac scintillation counter (Wallac, Gaithersburg, MD).

RESULTS

Differential Expression of BMP-2, BMP-4, and Type II BMP Receptor in Adult RPE; Lower Expression in Retinal Neurons

Database analysis (BLAST N, National Center for Biotechnology Information; Bethesda, MD) demonstrated that a clone from the RPE cDNA library isolated by differential hybridization had high homology to human BMP-4 and clones isolated by subtractive hybridization had high homology to BMP-2 or BMP-4. The probability scores for identity were very high (P = 7 × e⁻⁴⁻² for BMP-2 and P = 5 × e⁻⁴⁶ for the largest splice variant of BMP-4), suggesting that the clones represent bovine orthologs for the BMPs rather than novel BMP family members. Northern blot analysis using a labeled clone with homology to BMP-2 showed a 3.1-kb transcript in the lane containing bovine RPE RNA and no detectable signal in lanes containing RNA from several other tissues, whereas a labeled clone with homology to BMP-4 showed differential hybridization to two transcripts of approximately 1.5 and 1.9 kb in RPE RNA (Fig. 1).

In situ hybridization with antisense BMP-4 riboprobes in albino mouse retinas showed a strong signal in the RPE (Fig. 2A, arrows) consistent with that observed in northern blot analysis. There was also staining of cells of the outer and inner nuclear layer and some cells in the ganglion cell layer that was clearly more intense than the background seen when hybridization was performed with sense probes (Fig. 2B).

The type II BMP receptor is required for BMP signaling. Hybridization with antisense probe for type II BMP receptor showed strong staining in the RPE (Fig. 2C, arrows) but also showed faint staining in the retinal neurons of each layer. There was more prominent signal in the inner segments of photoreceptors (small arrows) than in the cell bodies in the outer nuclear layer, which is typical of many mRNAs, and was also seen for BMP-4 mRNA (Fig. 2A, small arrows). There was also increased signal for both BMP-4 and type II BMP receptor mRNAs along the borders of the inner nuclear layer (Figs. 2A, 2C; arrowheads), which could be due to higher mRNA levels in particular cell types, or the appearance of increased signal may be related to some sort of positional effect. There was little background when hybridization was performed with sense probes (Figs. 2B, 2D).
Using RT-PCR with primers specific for BMP-4, a product of appropriate size was amplified from freshly isolated RPE RNA (Fig. 3, RPE in situ) and RNA from two different primary RPE cultures (158 and 156) and one RPE cell line (ARPE).

A band was also amplified from RNA isolated from retina, but not from RNA obtained from freshly isolated bovine aortic vascular endothelial cells. Type II BMP receptor mRNA was expressed in a pattern similar to BMP-4 mRNA, except that its expression was more variable in RPE cultures, showing a strong signal in some (158) and none in others (156). It was also expressed in retina and freshly isolated aortic endothelial cells (Fig. 3), but was not detectable in several types of cultured vascular endothelial cells (not shown).

**Pattern of Expression of BMP-4 mRNA in the Retina during Development**

To explore a possible role for BMP-4 in retinal development, in situ hybridization was performed at several different time points. With antisense probes, there was a detectable but low signal in neuroblasts and developing ganglion cells of P0 mice (Fig. 4). A similar hybridization signal was seen in essentially all retinal cell bodies at several subsequent time points throughout retinal development (P3, P5, and P7), but a stronger signal was seen in P10, P17, and P35 (adult) ganglion cells, cells of the inner nuclear layer, and photoreceptor inner segments. There was no detectable signal when hybridization was performed with sense probe (Fig. 2; S). These experiments were performed in pigmented mice, but, because pigmentation obscures reaction product in the RPE, we cannot comment on the developmental pattern of expression in the RPE. The absence of identifiable modulation of BMP-4 mRNA throughout retinal development provides no clue to its possible function, but persistent mRNA levels in adults suggest that BMP-4 has some function in the retina and/or RPE of adult animals.

**Modulation of BMP-4 mRNA Levels in Ischemic Retinopathy**

Modulation of expression in animal models can sometimes provide clues concerning the function of a protein, and therefore the expression of BMP-4 mRNA levels was investigated in a murine model of oxygen-induced ischemic retinopathy. Mice killed after exposure to 75% oxygen between P7 and P12 showed BMP-4 mRNA levels (Fig. 5, 0h) similar to those seen in P12 mice reared in room air (Fig. 5, P12). Mice removed from oxygen to room air for 6 hours or longer (Fig. 5; 6h, 12h, 24h, and 5d) showed a striking decrease in the signal for BMP-4 mRNA in all retinal cell layers compared with P12 or P17 control mice. Hybridization with sense probe showed no signal (S).
in the inner nuclear layer. Semiquantitative RT-PCR also showed a decrease in BMP-4 mRNA in ischemic retinas at several time points (Fig. 6; 6h, 24h, and 5d) compared with nonischemic retinas (Fig. 6; 0h, P12, and P17).

**Northern Blot Analysis for TGF-β2 in Retinas of Mice with Ischemic Retinopathy**

Because BMP-2 and -4 are members of the TGF-β superfamily, we investigated the level of TGF-β2 mRNA in the retinas of mice with ischemic retinopathy. Northern blot analysis for TGF-β2 with 10 μg of total retinal RNA from nonischemic retinas showed multiple transcripts the same size as previously reported for TGF-β2 in mouse mammary glands22 (Fig. 7). There was an increase in retinal mRNA level between P12 and P17 (lane 1 versus lane 2), and no definite difference between P17 mice exposed to normoxia and P17 mice that had been exposed to 5 days of hyperoxia (lane 2 versus lane 3). There was a striking decrease, however, in retinal TGF-β2 mRNA 6 hours after removal of mice from hyperoxia to room air, resulting in retinal ischemia (lane 4 versus lane 3).

**In Situ Hybridization for BMP-4 in Mice with Retinal Degeneration**

Mice homozygous for a mutation in the gene for the β subunit of phosphodiesterase (rd mice) undergo degeneration of photoreceptors that begins on P10 and is nearly complete by P21.23 Hybridization with antisense probe using retinas from P9 C3H rd mice showed expression of BMP-4 mRNA in all retinal cell layers that was no different from that in P9 wild-type C3H mice (Fig. 8A and B). At P14, in the midst of the degeneration in rd mice, there was a marked decrease in BMP-4 mRNA in the degenerating photoreceptors and cells of the inner retina (Fig. 8D) compared with that in wild-type mice (Fig. 8C). At P35, there was no signal for BMP-4 mRNA in the one remaining row of photoreceptors or in the other cells of the retinas.
the retina (Fig. 8F), whereas there was a good signal in all retinal cells of wild-type C3H mice (Fig. 8E).

To assess the effect of retinal degeneration on BMP-4 levels in RPE cells, in situ hybridization was performed on albino FVB
rd
mice. At P9, there was a strong signal for BMP 4 mRNA in the retina and RPE (arrowheads, Fig. 9). At P14, there was a much weaker signal in both the retina and RPE. In the RPE, there were some areas where staining could be identified (arrowheads) and other areas where it was very weak or undetectable (arrows). At P21, the signal for BMP 4 mRNA was very weak or undetectable throughout most of the RPE (arrows), with occasional focal areas where staining was still seen (arrowheads). At P35, there was no detectable signal for BMP 4 in the retina or RPE (arrows).

Recombinant BMP-2 and BMP-4 Inhibit Proliferation of Cultured RPE Cells

TGF-βs inhibit the proliferation of RPE cells. 20 To determine whether BMPs have a similar effect, RPE cells that have been shown to express type II BMP receptors were grown to confluence, downshifted to serum-free medium for 24 hours, and then incubated in recombinant BMP-2 or BMP-4 in the presence or absence of serum. Incubation in BMP-2 or BMP-4 alone resulted in no difference in [3H]thymidine incorporation compared with media alone, but in the presence of serum, each caused a significant decrease compared with cells incubated in serum without BMPs (Fig. 10). As noted previously, TGF-β had a similar effect.

DISCUSSION

In this study, clones with high homology to human or murine BMP-2 or BMP-4 were identified in an adult bovine RPE cDNA library. Northern blot analysis showed a high level of expression in the RPE compared with other tissues. Although expression in the bovine retina was not detectable by northern blot analysis using total RNA, it was detectable by RT-PCR and in situ hybridization in mouse retina. There was an increase in BMP-4 mRNA in several retinal cell types during the latter stages of retinal development, with expression maintained in adult retina and RPE. This suggests that BMPs have some function in adult retina and RPE.

In an attempt to find some clues to the function of BMP-4, we assessed its mRNA levels in the retina in two animal models. Hypoxia is a potent stimulus for neovascularization, and several genes induced by hypoxia have been implicated in the signaling cascade leading to neovascularization. 21–27 Rather than showing an increase in hypoxic retina, BMP-4 mRNA was dramatically decreased. To determine whether other members of the TGF-β superfamily are modulated in the same way, northern blot analyses for TGF-β2 were performed and showed that TGF-β2 mRNA is also decreased in ischemic compared with control retinas. This is intriguing because TGF-β2 inhibits endothelial cell proliferation and has been implicated as an endogenous inhibitor of retinal neovascularization. 28

Basic fibroblast growth factor and ciliary neurotrophic factor have been implicated as survival factors in the retina 29,30; their expression is increased during the early stages of retinal degeneration. 31 We wanted to determine whether this was also the case for BMP-4. However, BMP-4 mRNA was markedly decreased in the retina and RPE during and after degeneration of photoreceptors in rd mice.

Therefore, two very different types of insult to the retina each resulted in a striking decrease in BMP-4 mRNA. One feature that these insults have in common is that they result in a proliferative response in the retina. If BMP-4 acts as a growth regulator for one or more cells in the retina, then its down-regulation may be part of the reparative response allowing positive growth regulators to stimulate proliferation. To test this possibility, we first wanted to determine whether type II BMP receptors could be identified on two of the cell types

![Figure 6. RT-PCR for BMP-4 mRNA in ischemic mouse retinas. One microgram RNA from mouse retinas was used for RT-PCR with primers specific for BMP-4 or 16S. There was a decrease in BMP-4 mRNA in ischemic retinas (6h, 24h, or 5d) compared with normoxic (P12 and P17) or hyperoxic (0h) retinas.](https://example.com/f6)

![Figure 7. TGF-β2 mRNA levels in ischemic, normoxic, and hyperoxic mouse retinas assessed by northern blots. Ten micrograms total retinal RNA from control normoxic P12 or P17 mice, hyperoxic mice (0h), and mice with ischemic retinas (6h) was used in northern blot analysis using a probe specific for TGF-β2. There was a dramatic decrease in TGF-β2 mRNA in ischemic retinas compared with those from normoxic and hyperoxic retinas. This blot was repeated using samples from other mice and showed the same results.](https://example.com/f7)
involved in proliferative diseases, RPE cells and vascular endothelial cells. The mRNA for type II BMP receptors was identified in freshly isolated RPE cells and vascular endothelial cells and was also found in some RPE cultures, but not in several types of vascular endothelial cell cultures. Therefore, we were able to investigate the effect of recombinant BMPs on proliferation of cultured RPE cells, but not vascular endothelial cells. BMP-2 and BMP-4 each inhibited serum-induced proliferation of RPE cells, suggesting that the BMPs may act as negative growth regulators for RPE cells, as is the case for TGF-βs. 20

BMP-4 plays an important role in dorsal-ventral patterning in early embryonic development. 4,5 It inhibits neurogenesis and is antagonized by chordin 32,33 and noggin, 34 which bind to BMP-4, block its activity, and promote neurogenesis. Therefore, BMP-4 is one of a group of secreted antagonistic factors that regulate neural development. In late development, BMP-2 and -4 stimulate apoptosis in areas of the body where tissue regression occurs. 8,9 Although the mechanism by which they stimulate apoptosis is not known, they presumably antagonize other growth factors that act as survival factors. Our data suggest that in adult retina and RPE, BMP-4 may act antagonistically with positive growth regulators. Therefore, the effects of BMPs in several different settings may involve balancing the action of other factors with opposite activity.

RPE cells express BMPs and BMP receptors suggesting the possibility of a negative autocrine loop. The same situation exists for TGF-βs, and there are several autocrine loops involving positive growth regulators in RPE cells. 35 Therefore, growth regulation of RPE cells may occur by a complex pro-

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**FIGURE 8.** In situ hybridization for BMP-4 in rd mice at different stages of photoreceptor degeneration compared with age-matched mice with the same genetic background. Hybridization with antisense BMP-4 probe using retinas from P9 C3H rd mice (B) before the onset of photoreceptor degeneration, shows expression of BMP-4 mRNA in all retinal cell layers that was no different from that in P9 wild-type C3H mice (A). At P14, in the midst of the degeneration in rd mice, there was a marked decrease in BMP-4 mRNA in the degenerating photoreceptors and cells of the inner retina (D) compared with wild-type mice (C). On P35, there was no signal for BMP-4 mRNA in the one remaining row of photoreceptors or in the other cells of the retina (F), whereas there was a good signal in all retinal cells of wild-type C3H mice (E).

**FIGURE 9.** Photoreceptor degeneration resulted in decreased BMP-4 mRNA in RPE cells. Hybridization with antisense BMP-4 probe using retinas from P9 albino FVB rd mice before the onset of photoreceptor degeneration showed a good signal for BMP-4 mRNA in the retina and RPE (arrowbeads). At P14, in the midst of the degeneration, there was a marked decrease in BMP-4 mRNA in the retina and RPE. In the RPE, there were some areas where BMP-4 mRNA was detectable (arrowbeads) and other areas where there was little or no signal (arrows). At P21, there were a few focal areas where BMP-4 mRNA was detectable in the RPE, but elsewhere the staining was weak or undetectable (arrows). At P35, there was no detectable signal for BMP-4 mRNA in the RPE (arrows).
cess involving modulation of antagonistic paracrine and autocrine signals. The mechanism by which this occurs is not known, but it is likely that some master control signals that alter the gain on the various loops originate from the retina, because retinal detachment or photoreceptor degeneration releases RPE cells from a growth-inhibited state and makes them more responsive to growth stimulators. Retinal-derived BMPs, TGF-β, and retinoic acid are all candidates for this action, and the demonstration in this study that retinal degeneration results in decreased BMP-4 mRNA in the retina and RPE, supports the hypothesis that BMP-4 plays such a role. Because excessive proliferation of RPE cells is a central feature of proliferative vitreoretinopathy (PVR) and poor return of vision after retinal reattachment surgery, each of these agents, including BMPs, may be useful in the treatment and/or prophylaxis of PVR.

PVR is the major nonvascular proliferative retinopathy. Diabetic retinopathy and other ischemic retinopathies are the major types of vascular proliferative retinopathies in which retinal neovascularization occurs and often results in loss of vision. BMPs may also be involved in their pathogenesis. Vascular endothelial growth factor (VEGF) plays a major role in the stimulation of retinal neovascularization, but regulation of VEGF levels is unlikely to be the sole determinant of whether neovascularization occurs. There are patients with nonproliferative diabetic retinopathy or other retinal diseases who have elevated levels of retinal VEGF, yet no evidence of retinal neovascularization. Recently, we have demonstrated that rats with experimental autoimmune uveoretinitis have high levels of VEGF in the retina but also have high levels of TGF-β and no evidence of neovascularization, whereas rats with ischemic retinopathy have high levels of VEGF, low levels of TGF-β, and neovascularization. The demonstration in this study that TGF-β2 mRNA is decreased in ischemic retina is consistent with those data. Because TGF-β2 is known to antagonize the activity of growth stimulators for retinal vascular endothelial cells in vitro, it is conceivable that it has a similar role in vivo. We were not able to test directly the effect of BMPs on vascular endothelial cell growth, but the possibility that they collaborate with TGF-β in regulating blood vessel growth in the retina and RPE is worth investigating in the future.

References

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