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# Expression of bone morphogenetic proteins (BMP), BMP receptors, and BMP associated proteins in human trabecular meshwork and optic nerve head cells and tissues

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**Purpose:** Bone morphogenetic proteins (BMPs) are multi-functional cytokines that have wide ranging effects on a variety of cells and tissues. In the present study, we profile the expression of BMPs, BMP receptors, and BMP associated proteins in the human trabecular meshwork (TM) and optic nerve head (ONH), two tissues involved in glaucoma pathogenesis.
**Methods:** Total RNA was isolated and subjected to reverse transcriptase-polymerase chain reaction (RT-PCR) to examine the expression of BMP, BMP receptor, and BMP associated proteins in tissues and cultured cells from the human TM and ONH (ONH astrocytes and lamina cribrosa cells). Western immunoblotting was used to study the expression of BMP and BMP receptor proteins in cultured human TM and ONH cells.
**Results:** Both TM and ONH cells and tissues expressed mRNAs for BMP2, BMP4, BMP5, BMP7, BMP-RIA, BMP-RIB, BMP-RII, DRM(gremlin), follistatin, chordin and NMA (BAMBI). The proteins for BMP2, BMP4, BMP5, BMP7, and all three BMP receptors were expressed in cultured human TM and ONH cells.
**Conclusions:** Members of the BMP family of genes, including BMPs, BMP receptors, and inhibitory BMP associated proteins are expressed in the human TM and ONH. These molecules may be involved in the normal formation and function of these tissues. Altered expression of members of this gene family may lead to functional changes in the TM and ONH. These genes and their respective signaling pathways merit further research to examine their possible role in glaucoma.

Glaucoma is a heterogeneous group of optic neuropathies afflicting over 67 million individuals worldwide and is a major cause of irreversible blindness [1]. Three ocular tissues are effected in glaucoma pathogenesis. Alterations in the trabecular meshwork (TM) are associated with the development of impaired aqueous humor outflow [2-5], and the resulting ocular hypertension is a major risk factor for the development of glaucomatous optic neuropathy [6]. Loss of TM cellularity [7,8], the accumulation of extracellular material [2,9,10], and TM cell cytoskeletal changes [11] have been shown to occur in the glaucomatous TM. A hallmark of glaucoma is cupping and excavation of the optic nerve head (ONH), which is associated with the collapse and remodeling of the lamina cribrosa (LC) [12-16]. ONH changes are associated with inhibited retrograde transport of materials, including neurotrophic factors, in retinal ganglion cell axons [17,18] and atrophy of retinal ganglion cells. Retinal ganglion cells die by apoptosis [19,20] leading to loss of vision and characteristic visual field abnormalities.

Despite years of intensive research, the precise molecular mechanisms responsible for glaucomatous damage to the eye are not known. Recent research has suggested that growth factors may be important in maintaining normal homeostasis in the ocular tissues associated with glaucoma, and alterations in growth factor/growth factor receptors may play a role in glaucoma pathogenesis. Growth factors are a very large family of polypeptides that control cell growth and differentiation. These molecules have a variety of cell-specific effects on gene expression, extracellular matrix composition and deposition, cytoskeletal organization, and regulation of cellular functions. The TM expresses a wide variety of growth factors, growth factor receptors [21-26] as well as neurotrophin/neurotrophic factors and their receptors [27,28]. ONH astrocytes and lamina cribrosa cells, two cell types of the optic nerve head, express growth factors, neurotrophins and their receptors [29,30]. The aqueous humor also contains a variety of growth factors including FGF2, EGF, TGFβ, HGF [31-34] as well as neurotrophins [35]. Elevated levels of aqueous humor TGFβ-2 and HGF have been reported in primary open-angle glaucoma (POAG) patients [34,36-38]. Growth factors may be involved in glaucoma by altering the normal development and/or function of the TM and ONH.

Bone morphogenetic proteins (BMPs) are an interesting family of growth factors originally identified as osteoinductive cytokines that promote bone and cartilage formation, but they also have recently been shown to be involved in development, morphogenesis, cell proliferation, and apoptosis of a wide variety of tissues and cells [35,39,40]. BMPs are members of the TGFβ superfamily, and there are approximately 15-20

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BMPs genes in man, 3 BMP receptors, and a number of BMP associated proteins that function as BMP antagonists [41]. BMPs and BMP receptors are expressed in ocular tissues [42,43], and BMP2, BMP4, and BMP7 are involved in the development of the lens and retina [44-47]. BMP6 and BMP7 also appear to play a role in protecting neurons from hypoglycemic or ischemic damage [48,49], and BMP2 has been shown to enhance ganglion cell neurotrophin expression [50].

BMPs may be involved in the normal functioning of the tissues involved in glaucoma pathogenesis, and alterations in their expression may be associated with the development of glaucoma. The purpose of the present study was to determine whether BMPs, BMP receptors and BMP binding proteins are expressed in human TM and ONH cells and tissues.

## METHODS

*Cell culture:* Human trabecular meshwork cells were generated as described previously [11,25-28,51-57]. TM cells were grown from TM explants of donors aged 6 days, 6 months, 2, 54 and 80 years. Human optic nerve head astrocytes and lamina cribrosa (LC) cells were generated from carefully dissected optic nerve heads (donors aged 2 days to 90 years) and characterized according to previous reports [29,58]. The cells were grown to confluency in the following media: Ham's F10 media (JRH Biosciences, Lenexa, KS) containing 10% fetal bovine serum (HyClone, Logan, UT) and antibiotics (Gibco BRL-Life Technologies, Grand Island, NY) for TM cells; Dulbecco's modified Eagle's media (DMEM, HyClone) containing 10% FBS for LC cells; and astrocyte growth medium (AGM, Clonetics, San Diego, CA) containing 5% FBS.

*RT-PCR:* Total cellular RNA was prepared from cultured TM and ONH cells and tissues using TRIzol extraction (Gibco BRL-Life Technologies), and first strand cDNA synthesis was done as previously reported [25,26,28,29]. Primers for the various BMP, BMPR, and BMP binding proteins were designed using Oligo 4.0 software (National Biosciences, Plymouth, MN). The primer pairs, expected product sizes, and annealing temperatures are listed in Table 1. All primer pairs were designed so that amplification of potentially contaminated genomic DNA sequences would produce mRNA PCR products that would be substantially larger than expected, because intron sequences that were excised during RNA processing would be included in genomic DNA. The β-actin PCR primers, AGGCCAACCGCGAGAAGATGACC (upstream) and GAAGTCCAGGGCGACGTAGCAC (downstream) with an annealing temperature of 55 °C yielded a PCR product of 350 bp. PCR and gel electrophoresis were done as previously reported [25,26,28,29]. To ensure specificity of the RT-PCR products, Southern blot analysis was performed with probes designed using Oligo 4.0 that hybridized to a region within the amplified PCR product.

**TABLE 1. PCR PRIMER PAIRS**

| Gene | Accession Number | Upstream Primer (5'-3') | Downstream Primer (5'-3') | Size (bp) | Annealing Temp (°C) |
|---|---|---|---|---|---|
| BMP-2 | NM-001200 | GGAAGAACTACCAGAAACGAG | AGATGATCAGCCAGAGGAAAA | 657 | 55 |
| BMP-4 | NM_001202 | ACCTGAGACGGGGAAGAAAA | TTAAAGAGGAAACGAAAAGCA | 348 | 55 |
| BMP-5 | NM_021073 | AAGAGGACAAGAAGGACTAAAAATAT | GTAGAGATCCAGCATAAAGAGAGGT | 303 | 55 |
| BMP-7 | NM_001719 | AGCCCGGGTAGCGCGTAGAG | GCGCCGGTGGATGAAGCTCGA | 202 | 60 |
| BMP-RIA | NM_004329 | TAAAGGTGACAGTACACAGGAACA | TCTATGATGGCAAAGCAATGTCC | 298 | 55 |
| BMP-RIB | NM_001203 | TACAAGCCTGCCATAAGTGAAGAAGC | ATCATCGTGAAACAATATCCGTCTG | 211 | 55 |
| BMP-RII | NM_001204 | TCCTCTCATCAGCCATTTGTCCTTC | AGTTACTACACATTCTTCATAG | 457 | 55 |
| Cer-1 | NM_005454 | ATAGTGAGCCCTTCCCACCT | AATGAACAGACCCGCATTTC | 294 | 60 |
| Chordin | AF209930 | CTCTGCTCACTCTGCACCTG | CCGGTCACCATCAAAATAGC | 198 | 60 |
| DRM (Gremlin) | NM_013372 | ATCAACCGCTTCTGTTACGG | ATGCAACGACACTGCTTCAC | 197 | 60 |
| Follistatin | NM_006350 | TGCCACCTGAGAAAGGCTAC | ACAGACAGGCTCATCCGACT | 201 | 60 |
| Noggin | NM_005450 | CACTACGACCCAGGCTTCAT | CTCCGCAGCTTCTTGCTTAG | 212 | 60 |
| NMA (BAMBI) | XM_005791 | GATCGCCACTCCAGCTACATC | GGGCACGGCAATGACC | 471 | 58 |

Table 1 describes the expected sizes of PCR amplification products with each human BMP and BMP receptor primer pair and optimal annealing temperature. Primers were designed using Oligo 4.0 (National Biosciences, Plymouth, MN).

*Western immunoblotting:* Protein was extracted from cultured cells using lysis buffer, and proteins were separated by denaturing polyacrylamide gel electrophoresis prior to electrophoretic transfer to nitrocellulose membranes as described previously [29]. The membranes were blocked with 5% milk (for BMPs) or 3% gelatin (for BMPRs) and incubated with the following primary antibodies: BMP2, BMP4, BMP5, BMP7 (all from Santa Cruz, Santa Cruz, CA), or BMP-RIA, BMP-RIB, BMP-RII (from Jackson Immuno Research, West Grove, PA). The membranes were washed, incubated with secondary antibodies (goat anti-mouse IgG-horseradish peroxidase for BMPs, Santa Cruz; donkey anti-goat-horseradish peroxidase for BMP receptors, Jackson Immuno Research), and developed using the WesternBreeze chemiluminescence immunodetection system (Invitrogen, Carlsbad, CA).

## RESULTS

*Expression of BMP and BMP receptor mRNA in human trabecular meshwork cells and tissues:* Amplification products

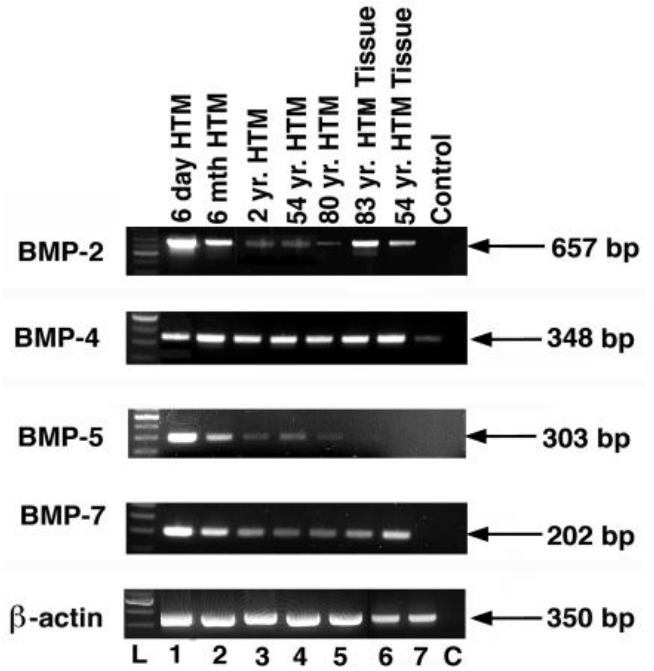


Figure 1. BMP expression in human TM cells and tissues. Ethidium bromide-stained agarose gel of BMP PCR products from cDNA samples generated from RT-PCR analysis of BMP expression in human trabecular meshwork cells (lanes 1-5) and tissues (lanes 6-7). L = base pair markers. C = PCR negative control lane. β-actin was used as a positive RT-PCR internal control.

of expected size for BMP-2, BMP-4, BMP-5 and BMP-7 primer pairs in human trabecular meshwork (TM) cells and tissues are shown in Figure 1. Southern blots using specific probes verified that these were the expected PCR products (data not shown). All human TM cell lines and tissues expressed message for BMP-2, BMP-4, and BMP-7. However, message for BMP-5 was low to undetectable in human TM tissue samples (Figure 1, lanes 6 and 7). Control reactions without cDNA did not result in amplification products indicating that reagents and primers were free of DNA or RNA contamination (Figure 1, lane C).

Figure 2 shows the amplification products of expected size for BMP-RIA, BMP-RIB, and BMP-RII primer pairs in human TM cells and tissues. All human TM cells and tissues expressed message for the BMP receptor complexes. Southern blots using specific probes verified that these were the expected PCR products (data not shown). An alternate amplification product (350 bp) was detected in the BMP-RII PCR reaction. The alternate amplification product was present in all human TM cells and tissues. This alternate band is currently being identified to determine if it is an alternate spliced form of the receptor. Control reactions without cDNA did not result in amplification products (Figure 2, lane C) indicating that reagents and primers were free of DNA or RNA contamination.

*Expression of BMP and BMP receptor mRNA in human ONH cells and tissues:* Amplification products of expected size for BMP-2, BMP-4, BMP-5, and BMP-7 primer pairs in human ONH astrocytes and ONH tissues are shown in Figure 3. All ONH astrocytes and ONH tissue expressed message for the respective BMP. Human brain astrocytes were used as a positive control cell line. Southern blots using specific probes verified that these were the expected PCR products (data not shown). With the exception of BMP-2, all other BMP were expressed by human brain astrocytes (Figure 3, lane 7). Control reactions without cDNA did not result in amplification products (Figure 3, lane C) indicating that reagents and primers were free of DNA or RNA contamination.

Figure 4 shows the amplification products of expected sizes for BMP-2, BMP-4, BMP-5, and BMP-7 primer pairs in cultured human LC cells. All LC cell lines expressed message for each BMP. Southern blots using specific probes verified that these were the expected PCR products (data not shown). Control reactions without cDNA did not result in amplification products (Figure 4, lane C) indicating that reagents and primers were free of DNA or RNA contamination.

Amplification products of expected size for BMP-RIA, BMP-RIB, and BMP-RII primer pairs in human ONH astrocytes and ONH tissues are shown in Figure 5. All ONH astrocyte cell lines and tissues expressed message for BMP-RIA and BMP-RIB. Southern blots using specific probes verified that these were the expected PCR products (data not shown). With the exception of ONH tissue (Figure 5, lane 6), BMP-RII was expressed by all ONH astrocyte cell lines. Message

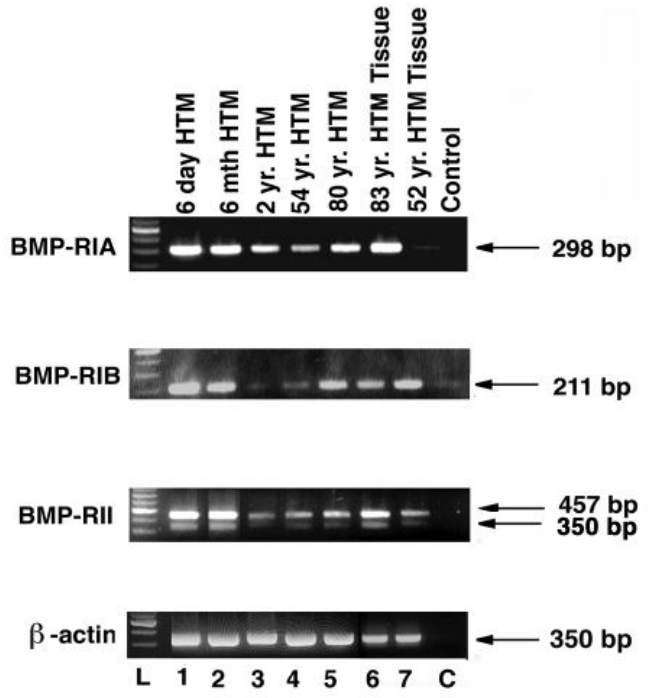


Figure 2. BMP receptor expression in human TM cells and tissues. Ethidium bromide-stained agarose gel of PCR products from cDNA samples generated from RT-PCR analysis of BMP receptor expression in human trabecular meshwork cells (lanes 1-5) and tissues (lanes 6-7). L = base pair markers. C = PCR negative control lane. β-actin was used as a positive RT-PCR internal control.

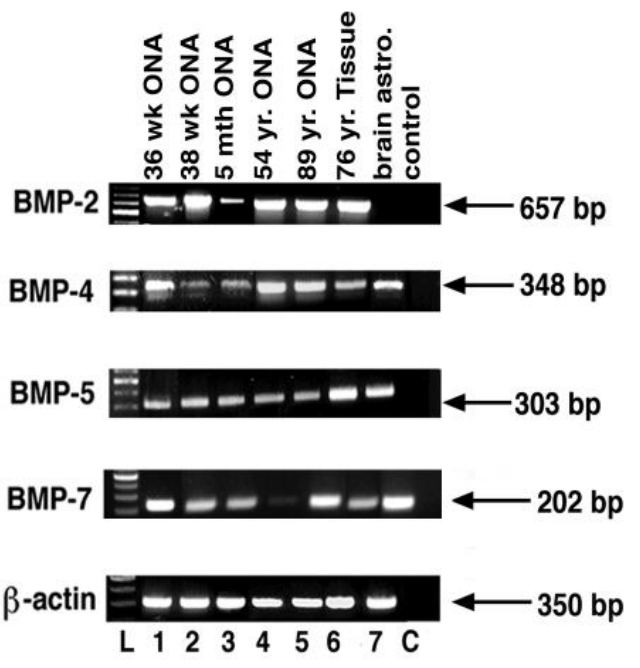


Figure 3. BMP expression in human ONH astrocytes, ONH tissues, and human brain astrocytes. Ethidium bromide-stained agarose gel of PCR products from cDNA samples generated from RT-PCR analysis of BMP expression in human ONH astrocytes (lanes 1-5), ONH tissue (lane 6), and human brain astrocytes (lane 7). L = base pair markers. C = PCR negative control lane. β-actin was used as a positive RT-PCR internal control.

for all BMP receptors (Figure 5, lane 7) was expressed by a human brain astrocyte cell line that served as a positive control. There appears to be a discrepancy in the expression of BMP-RII in ONH tissue and ONH cell lines. The reduced expression in ONH tissue may reflect a low level of expression. Control reactions without cDNA did not result in amplification products (Figure 5, lane C) indicating that reagents and primers were free of DNA or RNA contamination.

Figure 6 shows the amplification products of expected size for BMP-RIA, BMP-RIB, and BMP-RII primer pairs in cultured human LC cells. All LC cell lines expressed message for each BMP receptor. Southern blots using specific probes verified that these were the expected PCR products (data not shown). Control reactions without cDNA did not result in amplification products (Figure 6, lane C) indicating that reagents and primers were free of DNA or RNA contamination.

*Expression of BMP proteins and BMP receptor proteins in human TM and ONH cells and tissues:* Figure 7 represents chemiluminescent immunoblot detection of BMP-2, BMP-4, BMP-5, BMP-7, BMP-RIA, BMP-RIB, and BMP-RII proteins in human TM and ONH cells and tissues. All cell lines studied expressed the respective BMP proteins. The BMP proteins were detected in cell lines the following molecular weights: 54-56 kDa for BMP2, 25-27 kDa for BMP4, 55-57 kDa for BMP5, and 77 kDa for BMP7. Multiple bands were detected for BMP2 and BMP4, which most likely represent glycosylated, and partially glycosylated forms of these BMPs as seen in other studies. However, we did not do glycosylation studies as they were beyond the scope of this study. The BMP receptor proteins were detected in cell lines at molecular weights: 38 kDa for BMP-RIA, 64 kDa for BMP-RIB, and 57 kDa for BMP-RII. Multiple bands were detected for BMP-RIB and BMP-RII in the TM cells, which most likely represent glycosylated, and partially glycosylated forms as seen in other studies. As indicated above, studies to demonstrate if these products were glycosylated were not performed. The expression levels of proteins for the BMP receptors appeared to be lower in the TM cells compared to ONH cells. For example BMP-RII was not detected in TM cells and BMP-RIB was greatly reduced.

*Expression of BMP associated protein mRNAs in cultured human TM cells and in human ONH cells:* Amplification products of expected size for BMP associated protein primer pairs in human TM cell lines are shown in Figure 8. Human TM cell lines expressed message for DRM (gremlin), chordin, follistatin, and NMA (BAMBI). Southern blots using specific probes verified that these were the expected PCR products (data not shown). There was no apparent difference in message expression between cell lines. All human TM cells examined failed to express mRNA for the BMP associated proteins noggin and Cer-1 (Data not shown). Control reactions without cDNA did not result in amplification products indicating that reagents and primers were free of DNA or RNA contamination.

Amplification products of expected size for BMP associated protein primer pairs in ONH astrocytes and LC cell lines are shown in Figure 9. All ONH astrocytes and LC cell lines

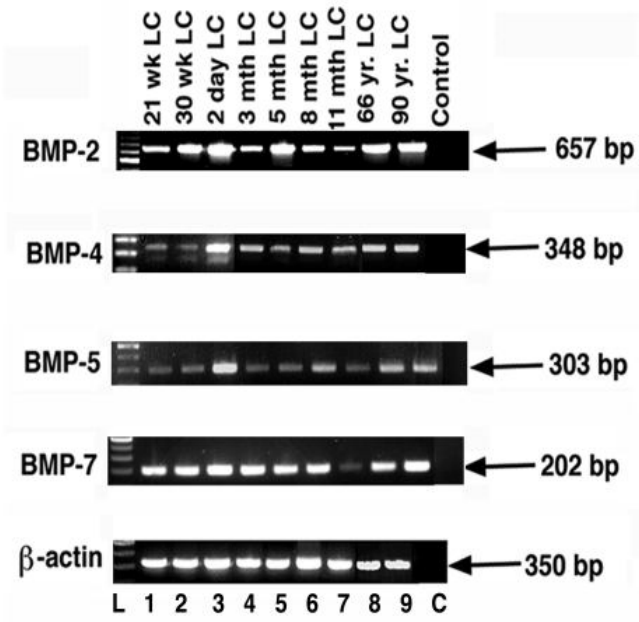


Figure 4. BMP expression in human lamina cribrosa cell lines. Ethidium bromide-stained agarose gel of PCR products from cDNA samples generated from RT-PCR analysis of human lamina cribrosa cells (lanes 1-9). L = base pair markers. C = PCR negative control lane. β-actin was used as a positive RT-PCR internal control.

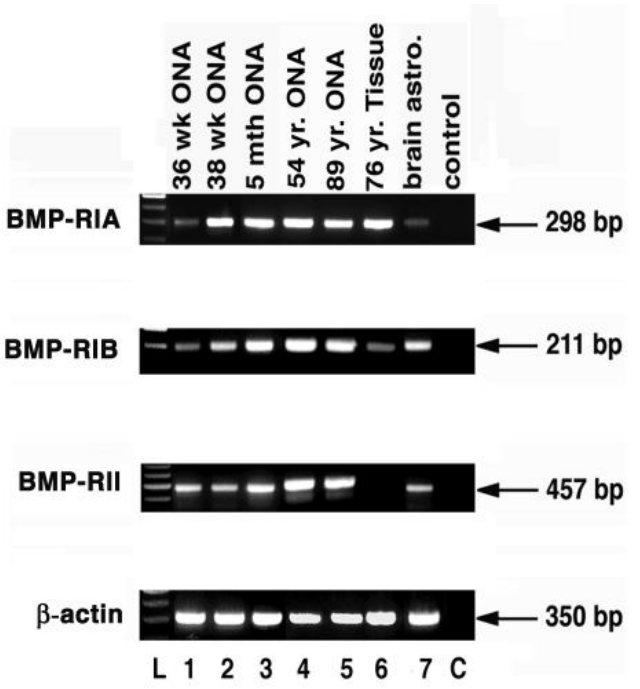


Figure 5. BMP receptor expression in human ONH astrocytes, ONH tissues, and human brain astrocytes. Ethidium bromide-stained agarose gel of PCR products from cDNA samples generated from RT-PCR analysis of BMP receptor expression in human optic nerve head astrocytes (ONA) (lanes 1-5), ONH tissue (lane 6), and human brain astrocytes (lane 7). L = base pair markers. C = PCR negative control lane. β-actin was used as a positive RT-PCR control.

expressed message for DRM (gremlin), follistatin and NMA (BAMBI). Southern blots using specific probes verified that these were the expected PCR products (data not shown). The majority of LC cells and ONH astrocytes expressed message for chordin. All human ONH astrocytes and LC cells examined failed to express mRNA for the BMP associated proteins noggin and Cer-1 (Data not shown). Control reactions without cDNA did not result in amplification products indicating that reagents and primers were free of DNA or RNA contamination.

## DISCUSSION

In this study we examined both the mRNA and protein expression of selected BMPs and members of the BMP receptor complex by human TM, ONH astrocytes and lamina cribrosa (LC) cells. In addition, we examined mRNA expression of BMP associated proteins. To our knowledge, this is the first report of the expression of BMPs, BMP receptors, and BMP associated proteins by cells and tissues from the human TM and ONH.

Bone morphogenetic proteins were initially identified as components of bone extracts that induced ectopic cartilage and bone formation [59]. In addition to bone and cartilage, BMPs and BMP receptors have been reported to be present in and/or act upon a variety of organs including the ovary [60], brain [61], epididymis [62], pancreas [63], breast [64], and kidney [44,46]. These proteins have now been shown to regulate many fundamental biological processes including cell proliferation, differentiation, apoptosis, cell migration, cell adhesion, and embryonic development [39]. The BMPs belong to the transforming growth factor-beta (TGFβ) superfamily, which also includes TGFβ-1, TGFβ-2, TGFβ-3, activins, and Mullerian inhibiting substance [65]. The BMPs form the largest subfamily within the TGFβ superfamily of growth factors. More than 15 BMP family members are expressed in mammals and *Drosphilia* [41,66]. BMPs are disulfide-linked dimer proteins containing seven cysteine residues that are conserved in the TGFβ superfamily [40].

Our studies are not the first to report BMP or BMPR expression in ocular tissues; however, most previous reports have focused on ocular development. The function of BMPs is important in ocular development since targeted disruption of genes encoding BMPs in mice leads to severe developmental defects in the retina and the lens [44,67,68]. There has been very limited information published concerning the role of BMPs in the human postnatal eye. Mohan and colleagues [69] reported that BMP-2 and BMP-4 and BMP receptors were expressed in cells of the adult cornea and suggested that BMP function might include corneal keratocyte proliferation and apoptosis. You and colleagues [43] verified this study and also reported the expression of BMP-3, BMP-5, and BMP-7 in ex vivo and cultured corneal epithelium and stromal cells. They reported that the level of BMP transcription was higher in the

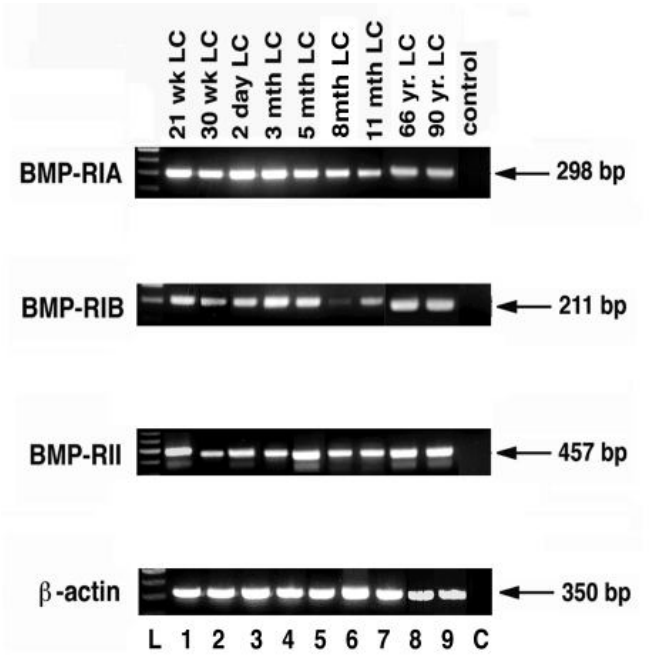


Figure 6. BMP receptor expression in human lamina cribrosa cell lines. Ethidium bromide-stained agarose gel of PCR products from cDNA samples generated from RT-PCR analysis of human lamina cribrosa cells (lanes 1-9). L = base pair markers. C = PCR negative control lane. β-actin was used as a positive RT-PCR control.

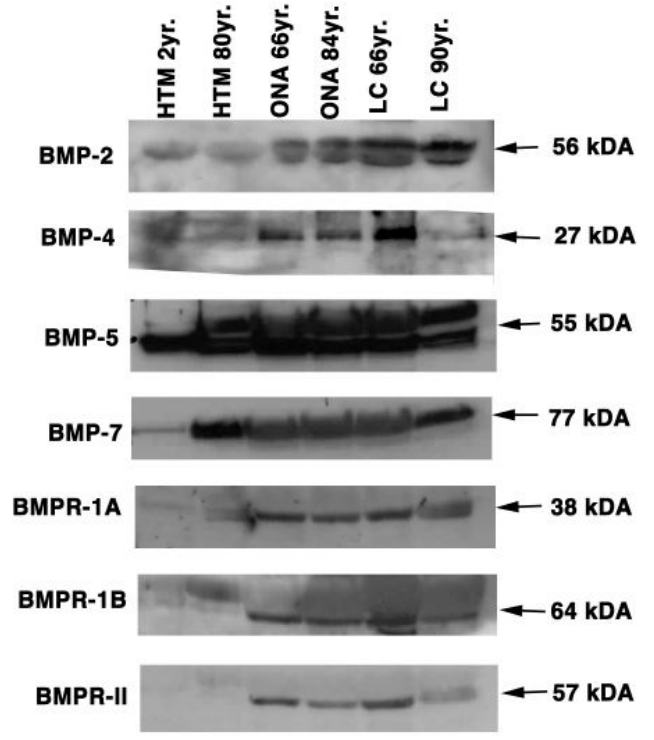


Figure 7. Western immunoblot of BMP and BMP receptor expression in cultured human TM cells, optic nerve head astrocytes (ONA), and lamina cribrosa cells. Chemiluminescent detection of BMP proteins and BMP receptors in human trabecular meshwork cells (lanes 1-2), ONH astrocytes (lanes 3-4), and lamina cribrosa cells (lanes 5-6). Protein size indicated in kDa.

stroma while the level for receptors was higher in cultured corneal epithelial cells.

Of particular importance with respect to BMPs and ocular development is the recent report by Chang and colleagues [70], which showed a heterozygous deficiency of *Bmp-4* resulted in anterior segment dysgenesis and elevated intraocular pressure. The abnormalities were similar to those in human patients with developmental glaucoma. Interestingly, the penetrance and severity of the abnormalities was strongly influenced by genetic background. The C57BL/6J background had the most severe abnormalities including the absence of the optic nerve. Thus BMP-4 may be involved in developmental conditions associated with human glaucoma.

While the majority of reports concerning the function of BMPs involve their trophic nature in the musculoskeletal system, numerous studies have established non-osseous tissues as targets for BMPs. For example, BMPs have protective effects in the adult central nervous system, and this action may be significant with respect to the human optic nerve head. Liu and colleagues [49] reported that BMP-7 had an effect on functional recovery, glucose utilization and blood flow following transient focal cerebral ischemia in rats. Since ischemia in the ONH has been suggested to play a role in glaucoma, our results that ONH astrocytes and lamina cribrosa cells express mRNA and protein for BMP-7 may be significant. In addition, BMP-7 provides a neuroprotective function in the adult CNS [71]. Interestingly, recent studies indicate that mRNA for BMP receptors is upregulated after brain injury [72]. It would be interesting to determine if BMP-RI and BMP-RII are upregulated in the glaucomatous ONH. Another potential action of BMP in the ONH may involve glial cell proliferation. ONH astrocytes are activated in glaucoma. It also has been reported that BMP-7 stimulates proliferation of astrocytes in vitro [73] as well as the differentiation of oligodendroglial-astroglial progenitor cells into astrocytes [74].

BMP signaling mechanisms are depicted in Figure 10. Signaling is initiated upon BMP binding to the cell-surface serine-threonine kinase receptors BMP-RI and BMP-RII. The BMP signal is propagated by Smads through protein-protein and protein-DNA interactions [75]. In the BMP-Smad pathway, BMP dimers bind the receptor complex, leading to phosphorylation of the type 1 receptor (BMP-RIA/BMP-RIB) by the type 2 receptor (BMP-RII) that in turn phosphorylates appropriate regulatory Smads (Smad1/Smad5) [76]. This allows the regulatory Smads to interact with the co-Smad (Smad4), and this complex enters the nucleus to activate or repress target gene expression, depending on which nuclear co-factors are present. Our results indicate that human ONH astrocytes and lamina cribrosa cells express message and protein for the BMP receptor complex. Thus these cells could respond to endogenous BMP ligands.

Recent reports indicate that regulation of BMP signaling can occur at the level of ligand availability. For example, BMP associated proteins can act as BMP antagonists. Examples of BMP associated proteins include noggin, chordin, follistatin, and members of the DAN (Differential screening-selected gene Aberrative in Neuroblastoma) family including cerberus, caronte, gremlin (Drm), and Dan. It is thought that BMP antagonist proteins bind directly to BMPs and prevent the ligand from interacting with the receptor complex [76]. Thus BMP

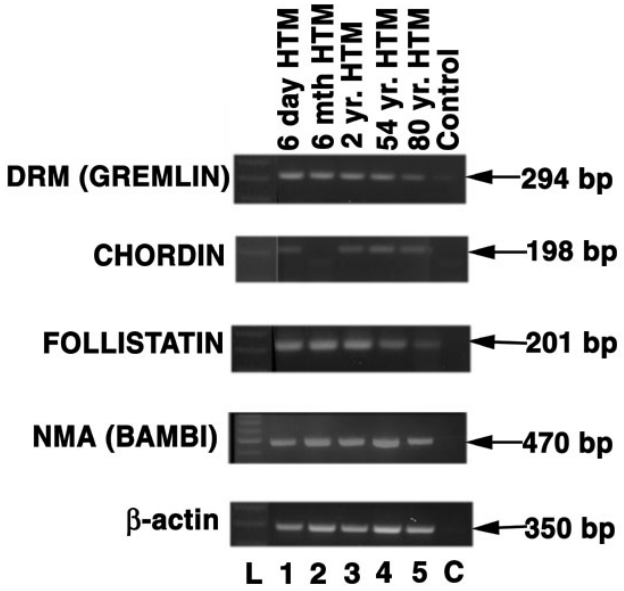


Figure 8. BMP associated protein mRNA expression in human TM cells. Ethidium bromide-stained agarose gel of PCR products from cDNA samples generated from RT-PCR analysis of human trabecular meshwork cells (lanes 1-5). L = base pair markers. C = PCR negative control lane. β-actin was used as a positive RT-PCR internal control.

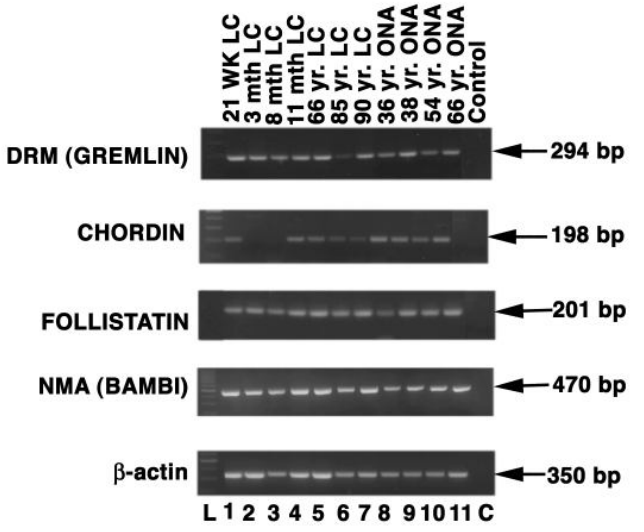


Figure 9. BMP associated protein mRNA expression in human lamina cribrosa cells and ONH astrocytes. Ethidium bromide-stained agarose gel of PCR products from cDNA samples generated from RT-PCR analysis of lamina cribrosa (LC) cells (lanes 1-7) and ONH astrocytes (ONA) (lanes 8-11). L = base pair markers. C = PCR negative control lane. β-actin was used as a positive RT-PCR internal control.

associated proteins act as antagonists and can directly control ligand bioavailability and the function of an individual BMP molecule at the cellular level. BMP associated proteins have been previously reported in a variety of cells. For example, Topol and colleagues [77] have shown that both gremlin and Dan (a) can bind and block the action of BMP-2 and/or BMP-4; (b) are highly expressed in non-dividing and differentiated cells such as neurons, type I alveolar cells, and goblet cells; and (c) can repress cell growth.

Our results demonstrate the expression of mRNA for BMP associated proteins follistatin, gremlin, chordin, and NMA (BAMBI) in cells of the human trabecular meshwork and optic nerve head. Thus BMPs and BMP associated proteins may reciprocally regulate dynamic cellular interactions. These results add an additional control mechanism of BMP signaling within these cells. For example, an alternative approach to controlling BMP signaling may involve the regulation of BMP associated proteins. Since these proteins are negative regulators of BMP ligands, a reduction in endogenous levels of BMP associated proteins will achieve a functional upregulation of BMP signaling. This aspect of cell signaling within the BMP family has not received extensive study but should be a future research activity.

While the majority of BMP associated proteins act by binding directly to BMP molecules, one BMP associated protein acts via a different mechanism. The BMP associated protein BAMBI (human ortholog NMA) is a transmembrane protein that serves as a dominant negative receptor of the functional BMP receptor complex directly inhibiting BMP-RI activation [78]. *Bambi* lacks an intracellular kinase domain, and it stably associates with TGFβ family receptors and inhibits BMP and activin as well as TGFβ signaling. The intracellular domain that prevents the formation of the receptor complexes mediates these inhibitory effects [78]. Interestingly, Grotewold et al. [79] demonstrated that *Bambi* is strictly co-expressed with *Bmp-4* during mouse embryogenesis and suggests that *Bmp-4*/*Bambi* forms a synexpression group. The presence of NMA (BAMBI) mRNA expression by cells of the human trabecular meshwork and optic nerve head provides additional flexibility in the regulation of BMP signaling in these tissues.

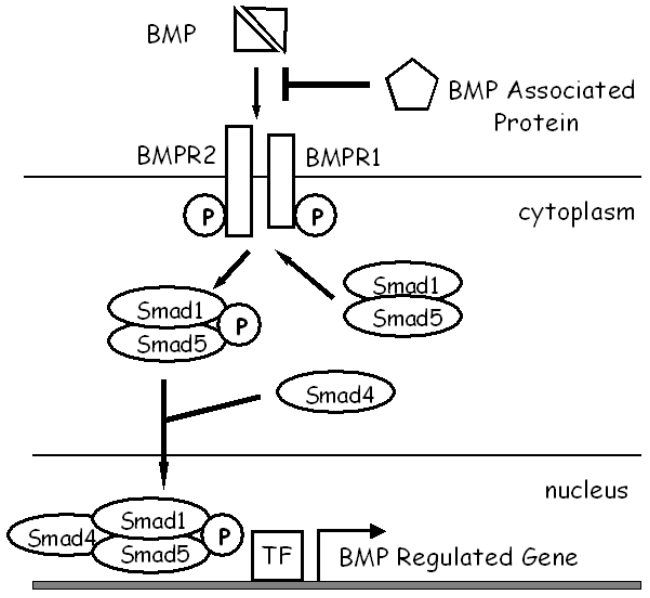


Figure 10. Bone morphogenetic protein signaling pathway. Bone Morphogenetic Protein (BMP) dimers bind to a membrane complex composed of BMP receptors 1 and 2, which are serine/threonine kinases. The regulatory Smads (Smad1/Smad5) become phosphorylated and associate with a co-Smad (Smad 4). This resulting Smad complex enters the nucleus where is associates with transcription factors (TF) and regulates gene expression. BMP associated proteins act as BMP antagonists by binding BMPs and preventing BMP interaction with BMP receptors.

In conclusion, human trabecular meshwork cells, human optic nerve head astrocytes, and lamina cribrosa cells express mRNA and protein for BMP-2, BMP-4, BMP-5, BMP-7 and the BMP receptor complex. In addition, these cells also express mRNA for BMP associated proteins. These results establish the BMP family of growth factors as potential important signaling molecules within the trabecular meshwork and optic nerve head. Future work will attempt to establish specific cellular functions associated with this family and to determine whether they play a potential role in glaucoma pathogenesis.

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