·Review·

Regulation of scleral fibroblast differentiation by bone morphogenetic protein-2

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Abstract

• **Bone morphogenesis proteins (BMPs) are multi-functional growth factors. They are expressed in retina, retinal pigment epithelium (RPE) and sclera and serve as a regulator in the growth and development of the eye. This article reviewed the chondrogenic potency of the sclera, biochemical and pathological changes of myopic scleral tissue and the differentiation of chondrogenesis by BMP-2. We proposed the hypothesis that BMP-2 can regulate differentiate of scleral fibroblasts and affect the development of myopia.**

• **KEYWORDS:** bone morphogenetic protein-2; sclera; myopia

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INTRODUCTION

Myopia is a highly prevalent ocular condition, the major symptom of which is blurred distance vision. The primary structural cause of myopia is increased axial length of the eye. Strong evidence from clinical and experimental studies indicates that the biochemical and biomechanical properties of the sclera determine the shape and size of the globe and therefore play a major role in influencing the refractive state of the eye. Significant scleral thinning and tissue loss, particularly at the posterior pole of the eye, were associated with ocular enlargement and myopia development after both short- and long-term treatments in a mammalian model of high myopia [1]. Investigation of postmortem highly myopic human eyes has revealed marked thinning of the sclera, particularly at the posterior pole, with changes in the scleral extracellular matrix (ECM)[2]. Similar changes have been observed in mammalian models of axial myopia, with marked thinning of the sclera at the posterior pole detected in monkeys and tree shrews[3,4].

The connective tissue of the sclera is comprised of ECM, including fibrillar collagens, proteoglycans, and small amounts of various glycoproteins, and matrix secreting fibroblasts.

Scleral biomechanical changes in pathological myopia are well documented both in humans and in animal models, with the sclera of myopic eyes demonstrating increased extensibility with increasing levels of myopia [5]. The major biochemical contributors to altered scleral biomechanics are reduced scleral collagen content, thinner collagen fibrils, and reduced amounts of sulfated and non-sulfated scleral glycosaminoglycans [6]. The overall effect of these changes is a weakened collagen matrix with increased internal stresses. Although ECM molecules previously believed unique to cartilage, such as aggrecan and proline arginine-rich end leucine-rich repeat protein (PRELP) have been identified in the human sclera, suggesting that cartilaginous components have been retained in the sclera through evolution and serve important biochemical and biomechanical functions [7]. Such findings have led to the hypothesis that thinning of the sclera in highly myopic eyes of human and animal models is a result of changes in scleral ECM metabolism[2].

The structural organisation of the sclera is largely reliant on the activity of the major ECM producing cell, the fibroblast. How the scleral fibroblasts monitor changes in the surrounding ECM and respond appropriately to chemical and mechanical changes in their environment is as yet unclear. Many aspects of scleral ECM remodeling are speculated to be under the control of specific growth factors. The mRNA levels of fibroblast growth factor receptor-1 (FGFR-1) have been shown to be up-regulated in tree shrew eyes developing myopia. It is speculated that increased levels of FGFR-1 on scleral fibroblasts may provide the potential target site for the action of exogenously applied b -FGF to regulate myopic eye growth. The finding that age-related changes in scleral proteoglycan synthesis rates in humans are nearly identical to that observed in articular cartilage, peaking in the fourth decade of life suggests that postnatal scleral growth, like that of other connective tissues, is under the control of growth hormone or its downstream effectors[7]

Bone morphogenetic proteins (BMPs) are the largest subfamily of the transforming growth factor-β and 30-38kDa

homodimers that are synthesized as precursor peptides of approximately 400-525 amino acids [8]. They have similar sequences including seven similarly-spaced cysteine residues located in the mature region of the proteins. To date, about 20 bone morphogenesis protein (BMP) family members have been identified and characterized[9].

BMPs are involved in numerous cellular functions including development, morphogenesis, cell proliferation, apoptosis, and ECM synthesis [10]. The mice lacking BMP-7 displayed severe defects confined to the developing kidney and eye. This showed that BMPs are essential for early morphogenesis of the eye[11]. BMP and BMP receptors are expressed by adult retinal pigmented epithelium (RPE), with BMP-2 and BMP-4 downregulated by injury, to allow tissue repair[12].

BMPs may play roles in refractive error and eye growth regulation. BMP2 gene expression in chick retina/RPE was down-regulated [13] in form-deprivation myopia, but it was significantly up-regulated when the eyes wearing +10D lens and significantly down-regulated when the eyes wearing -10D lens [14]. These expression patterns were also similar to those observed for BMP4 and BMP7 [15]. BMP-2 and BMPRs were expressed in both human scleral fibroblasts and human sclera [16]. BMP-2, which promoted cell proliferation, and elicited changes in matrix metalloproteinase-2 (MMP-2) and tissue inhibitor of metalloproteinase-2 (TIMP-2), might influence ECM synthesis[17].

Bone morphogenetic protein-2 (BMP-2) was originally described for its ability to induce the entire cascade of endochondral bone formation. BMP-2 is now recognized as a multipurpose cytokine that stimulates migration and induces differentiation of many different cell types [18]. In this article we review recent advances regarding the regulation of chondrogenic differention by BMP-2 and BMPs serves as a regulator in myopia development.

ORIGIN OF SCLERAL FIBROBLAST

The human sclera differentiates from neural crest and mesoderm in the sixth week of human embryonic development. The majority of the sclera differentiates from neural crest that surrounds the optic cup of neuroectoderm. However, a small temporal portion of the sclera differentiates from mesoderm which also contributes to the striated extraocular muscles and vascular endothelia [19,20]. Similar to the sclera, cartilage, bones, ligaments, tendons, dermis, leptomeninges, and perivascular smooth muscle differentiate from a dual origin of neural crest and mesoderm, so we can infer that human sclera has some similarities with cartilage, ligaments and tendons.

Although the human sclera is not a cartilaginous tissue, the human sclera maintains chondrogenic potential throughout evolution [21]. Cultured scleral cells exposed to TGF-β and BMP-2 produced an abundant matrix and the expression of cartilage-associated genes, such as Indian hedge hog, type X collagen, and MMP13, was up-regulated within 3 weeks *in vitro*. Sheep sclera may have characteristics in common with cartilage and that scleral cells may possess chondrogenic potential [22]. Scleral cartilaginous metaplasia was detected by routine histologic examination of globes from 5 Suffolk sheep. The matrix surrounding chondrocytes stained intensely with alcian blue and was immunopositive for type II collagen. Tsai *et al* [23] identified and cultured multipotent scleral stem/progenitor cells (SSPCs) from the murine sclera. These cells were positive for the mesenchymal markers Sca-1, CD90.2, CD44, CD105, and CD73 and negative for the hematopoietic markers. SSPCs were able to differentiate to adipogenic, chondrogenic, and neurogenic lineages. This indicated that the sclera contains multipotent mesenchymal stem cells. Further study of SSPCs may help elucidate the cellular and molecular mechanism of scleral diseases such as scleritis and myopia.

SCLERAL CHANGES DURING MYOPIA DEVELOPMENT

In humans, it has been established that the posterior sclera of high-myopic eyes becomes thinner as the axis of the eye elongates. Many morphological and biochemical studies indicate that ocular enlargement in the chick is due to active growth of the sclera[24].

This scleral thinning observed in highly myopic human eyes is associated with thinning of collagen fiber bundles as well as with a reduction in the size of the individual collagen fibrils with a preponderance of unusually small diameter fibrils averaging below 60-70nm [25]. McBrien *et al*[26] investigated that significant scleral thinning and tissue loss, particularly at the posterior pole of the myopic eye were associated with ocular enlargement and myopia development induced by monocular deprivation of pattern vision for short-term (12d) or long-term (3-20 months) periods. But collagen fibril diameter distribution was not significantly altered after short-term myopia treatment, whereas in the longer term, there was an increasing number of small diameter collagen fibrils in the sclera of highly myopic eyes, which is consistent with findings in humans. It was speculated that during the initial stages of myopia development, the mechanical properties of the sclera must be controlled by additional factors, such as proteoglycans. But during the later stages of myopia development, the increased numbers of small-diameter fibers, in conjunction with the reduced proteoglycan content of the tissue, contributed to a weakened biomechanical properties of the sclera that was less resistant to imposed mechanical stresses (such as intraocular pressure). These changes could result in elongation of axial length and the formation of posterior staphyloma. The mammalian sclera is predominantly composed of fibrillar collagens, proteoglycans, and small amounts of various glycoproteins. The measurement of

sulfate incorporation into glycosaminoglycans (GAG) polymers is a useful index of proteoglycan synthesis and has been used as a marker of scleral metabolism during the investigation of myopia development in both birds and mammals. McBrien [27] demonstrated a decrease in GAG synthesis in axial myopia development. Given the intrinsic role of GAGs in ECM biomechanics, hypothesis that scleral glycosaminoglycan content is a major factor underlying the early changes in the viscoelastic properties of the sclera of myopic eyes is reasonable. Many researchers have concluded that scleral ECM biochemical changes involved in myopic development. So some methods such as TGF-β, optical correction were introduced to regulate the metabolism of ECM of sclera to slow up or suspend the development of mypia[28,29].

Degradation of scleral connective tissue during remodeling is partially regulated by the balance between MMPs and TIMPs. Concurrent with an increase in eye size, both the hydrational capacity of the sclera and the proteolytic activities such as the activities of scleral serine proteinase and matrix metalloproteinase systems are increased in eyes relative to control eyes in a regional manner. Increases in the hydration capacity can be interpreted as resulting from decreases in the strength of the scleral collagen meshwork and increases in proteoglycan content. Elevation of proteolytic activities can lead to degradation of collagen meshwork[30].

It appears that a dynamic relationship exists between a cartilaginous layer and the fibrous layer found in the chick sclera during visual deprivation-induced growth; the cartilaginous sclera becomes thicker, while the fibrous sclera becomes thinner in the posterior segment of myopic eyes[31]. Kusakari *et al* [24] demonstrated that in the posterior segment of myopic eyes the border between the cartilaginous and fibrous layers was indistinct because of collagen bundles of the fibrous sclera that spread into the cartilaginous sclera, whereas in control eyes the distinction was clear. Moreover various types of transitional cells, from fibroblast-like mesenchymal cells to chondrocytes, were found in the border between the cartilaginous and fibrous layers. Collagen fibrillar diameters of the fibrous sclera in the posterior segment of myopic eyes were smaller than in control. Thus, changes in the fibrous sclera in myopic eyes of chicks seem to be similar to scleral changes in myopic eyes of mammals.

REGULATION OF CHONDROGENIC DIFFERENTIATION BY BMP–2

BMP–2 Regulating Stem Cells BMP-2 or BMP-4 induced embryonic stem (ES) into chondrogenic differentiation. as shown by the appearance of Alcian blue-stained nodules and expression of collagen II, cartilage oligomeric matrix protein (COMP) and the cartilage associated genes such as scleraxis, Pax-1, Sox 9, collagen II and aggrecan [32]. BMP-2 significantly promoted chondrogenic differentiation of synovium-derived stem cells (SDSCs) *in vitro*[33]. SDSCs can differentiate to a chondrocytic phenotype in chondrogenic medium containing TGF-β3 with or without BMP-2. Safranin O staining of the ECM was positive and the expression of collagen type II was detected. Cell pellets treated with TGF-β3 and BMP-2 were larger in diameter and weight, produced more GAGs, and expressed higher levels of collagen type II and other chondrogenic markers than medium with TGF-β3 alone.

The murine mesenchymal stem cell line C3H 10T1/2 can be induced to become both chondrogenic and osteogenic when culture conditions are supplemented with BMP. Over a 16-day time-course after the addition of 0, 80 or 250ng/mL BMP-7 to C3H10T 1/2 cells, two genes associated with the progression of chondrogenic differentiation (collagen types II and X) and four genes associated with osteogenic differentiation (collagen type I, osteopontin, osteocalcin, and bone sialoprotein) were examined. Both BMP-7 and BMP-2 induced C3H10T1/2 cells to undergo a sequential pattern of chondrogenic followed by osteogenic differentiation[34].

BMP–2 Involving Chondrogenesis The synovium-derived progenitor cells cultured under 3D conditions and treated with BMP-2 exhibited chondrogenic differentiation activities by expressing Sox9, collagen type II and aggrecan[35]. Sox9 is a transcriptional activator that binds to the promoters and activates transcription of collagen type II and aggrecan [36]. Additionally, these cells began to accumulate GAG and to express collagen type II protein when treated with BMP-2[37].

Kim *et al* [38] have demonstrated that murine iPS cells spontaneously differentiate into chondrogenic cells in vitro by the appearance of cartilage nodules and the expression of cartilage-associated genes and proteins with an efficiency comparable to that of murine ES cells. Kuboth *et al*[39] showed treatment of iPS cells with 10ng/ml BMP-2 resulting in a increase in Alcian blue-stained nodules.

RMD-I (a clonal cell line)established from the skeletal muscle of a 20-day fetal rat represents a morphologically homogeneous population of undifferentiated mesenchymal cells, expressing α -smooth muscle actin and type I collagen, but no cartilage-associated genes. RMD-1 cells formed colonies and showed chondrocyte-like features when the medium containing BMP-2 including a distinct morphological change into spherical cells, an increase in the levels of sulfated glycosaminoglycans, a decrease in type I collagen mRNA and the expression of cartilage-associated genes, including type II collagen, type IX collagen, aggrecan and alkaline phosphatase[40].

Low level adenoviral BMP-2 could augment neocartilage parameters *in vitro* and *vivo*. Ng *et al* [41] compared neocartilage with and without 1)supplemented serum-free medium [chondrocyte differentiation medium (CDM)], 2)

AdBMP-2 transduction, and 3)varying ratios (0.1-1) of transduced and juvenile human chondrocytes (jCh). AdBMP-2 and neocartilage growth in CDM were histologically superior and size to standard medium.

BMP-2 Regulate Differentiation of Fibroblast BMP-2 was found to be a potent promoter of the chondrogenic differentiation of the neonatal human dermal fibroblasts (nHDFs) [42]. Application of BMP-2 to nHDFs elevated aggrecan gene expression and increased collagen production rate. Human turbinate fibroblasts were apparently redirected toward chondrogenic phenotype *in vitro* culture system under specific conditions [43]. When turbinate fibroblasts were cultured in three-dimensional scaffolds (alginate sponge) with growth factors (TGF-1 and IGF-I) and co-cultured with septal chondrocytes, co-culture of fibroblasts and chondrocytes showed comparable expansion of cells and ECM to culture of chondrocytes only. So there is possibility that humane scleral fibroblast has the potential of chondrogenic differentiation.

BMP-2 might be able to promote humane scleral fibroblast proliferation and differentiation, as well as to help ECM synthesis potentially through classical Smad pathway. Wang *et al*[46] clarified that in form-deprivation myopia (FDM) eyes, cell proliferation increased significantly and more cells differentiated into myofibroblast when incubated with BMP-2. The expressions of collangen I, aggrecan, and phospho-smad1/5/8 significantly increased as well.

SUMMARY

The biochemical and biomechanical properties of the sclera determine the shape and size of the globe and therefore play a major role in influencing the refractive state of the eye. Scleral fibroblasts are involved in scleral remodeling, which occurs during axial elongation in myopia. The thinned posterior scleral in high myopia is associated with a general loss of collagen and aggrecan which accounting for most of scleral ECM [44,45]. Many aspects of scleral ECM remodeling and fibroblast proliferation are regulated by specific growth factors.

BMP-2 induces bone and cartilage formation, controls fibroblast apoptosis, and regulates ECM synthesis in many tissues such as bone and teeth. BMP-2 has the capability to differentiate humane scleral fibroblast into cartilage and product many cartilage associated protein remolding the sclera which is the mechanism of myopia.

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