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MMP-2 participates in the sclera of guinea pig with form-deprivation myopia via IGF-1/STAT3 pathway

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Abstract. – OBJECTIVE: To investigate the expression changes of MMP-2 (matrix metalloproteinases-2) mediated by IGF-1 (insulin-like growth factors-1) STAT3 (signal transducer and activator of transcription 3) pathway in the sclera of the form-deprivation myopia guinea pigs.

MATERIALS AND METHODS: Twenty-four three-week-old guinea pigs were randomly divided into 4 groups: group A (Control), B, C and D. Guinea pigs in group A were sacrificed after 21 days without any special treatment. Guinea pigs in group B were sacrificed 7 days after receiving stitch in the right eye. Guinea pigs in group C were sacrificed 14 days after receiving st the right eye. Guinea pigs in group D w rificed 21 days after receiving stitch in th ht eye. Eyeball refraction and axial length of g a pigs were measured before sacrifice. Eyeba guinea pigs were enucleated after sacrifice. T expressions of IGF-1, STAT3 an 2 in scle al tissue were detected by W

RESULTS: Axial length nsio d myopia appeared in the right of gui pigs in group B. The expressio IGF-1 MMP-2 in the sclera si nif i ased af 7 days of occlusion pare that in control group A ($p<0$ in the rig of group C, the axial pr tion and my forma- tion appeared lay occlusio The expressions of 1, S nd MMP-2 in sclera significantly increased red with that in group A .05). In the ri e of group D, the axi tension and myop formation occurre F-1, S 3 and MMP-2 in scleral significantly upre ted 21 days after occlusion ($p<$ Fu rmore different stages of depriv otein e essions of MMP-2 and IGF-1 in wer sitively correlated ($r =$ $p<0.$

C CLUSIO Form-deprivation of guinea p lead to in reased expressions of IGF-1, ST MP-2 in the sclera and myopia of g e expressions of IGF-1, STAT3 MMP-2 increased progressively over the of deprivation. Additionally, overexpression -2 mediated by IGF-1/STAT3 pathway in scle ight promote the formation of myopia.

Key Words:
Form-de yopia, Sclera, ix metalloproteinase-2 in-li wth factor-1.

Introduction

Myopia is a co on eye disease with a high in- ce worldwi n Australia, the incidence of m among o 17-year-old students is 42.7-59.1 on to its high incidence, it also severely threatens physical health of myopia patients in different stages. Mild to moderate stage could affect quality of life. Additionally, mp tions of severe myopia such as retinal detachment and macular hemorrhage could potentially lead to blindness. Therefore, it is crucial to find out an effective way to prevent myopia.

The formalization of animal or human eyes depends on the coordination and precise control of different parts of the eye. Appropriate visual stimulation in early stage is very important to the normal growth and formalization of the eyeball. During the neonatal development, disturbed vision-dependent feed-back by form deprivation might cause the axial length of eye prolong, thus leading to myopia, namely form-deprivation myopia (FDM). In 1977, Wiesel et al[3(6)] successfully established animal model of form-deprivation myopia[4-6]. The thinning of the sclera is an important feature after the form-deprivation myopia, which is mainly due to the remodeling of the scleral extracellular matrix, especially the dynamic remodeling of the posterior scleral tissue. This remodeling has been considered as a result of imbalance between the scleral extracellular matrix (ECM) synthesis and degradation, in which the matrix metalloproteinases (MMPs) play a crucial role. Given that type I collagen can degrade human scleral collagen, matrix metalloproteinase 2 (MMP-2) has attracted the attention

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of researchers among kinds of MMPs[7]. MMPs are enzymes that are widely involved in the degradation of extracellular matrix in animals and plants. They can degrade almost all extracellular matrix components except for polysaccharides and play an important role in embryonic development and tissue plasticity. Among them, MMP-2 is capable of degrading various kinds of collagen composition[8]. Extracellular matrix degradation and sclera remodeling in the formation of deprivation myopia depend on MMP-2 secreted by posterior pole scleral fibroblasts[9]. Signal transducer and activator of transcription-3 (STAT3), an upstream factor of MMP-2, plays an important role in the regulation of MMP-2 expression. Zhang et al[10] found that STAT3 can mediate extravascular fibroblast migration via regulating MMP-2 expression. This finding indicated that MMP2 regulated by STAT3 signaling pathway may be one of the mechanisms leading to remodeling of the fibrous tissue in posterior pole of the sclera.

Various myopia-related growth factors such as insulin-like growth factor 1 (IGF1) can induce activation of STAT3 signaling in guinea pig scleral fibroblasts cultured *in vitro*[11]. In recent [illegible] research focused on the mechanism of [illegible] growth and regulation as well as the role [illegible] 1 in eyeball growth. Insulin-like growth [illegible] (IGF), as a molecular signal, is regarded to [illegible] an important role in maintaining and controlli[illegible] cell growth, proliferation, diffe[illegible] matura- tion and regeneration. The s[illegible] con[illegible] of two polypeptide growth facto[illegible] GF-I a[illegible] GF-II), IGF receptors (IGF-IR a[illegible] F-IIR) growth factor bindin[illegible] rote[illegible]) and IGF protease[12]. Among t[illegible], insuli[illegible] growth fac- tors 1 (IGF-1) ca[illegible] mote cell pr[illegible]ion, dif- ferentiation, ma[illegible]as well as su[illegible]ess cell apoptosis. Ad[illegible]onal[illegible]F-1 can also promote growth and anabolism, de[illegible] blood sugar and regulate i[illegible]une system via [illegible]ating various growth [illegible]mones. It has been reported that there is a [illegible]ificant [illegible]relation between the IGF-1 ge[illegible] ma[illegible]e disea[illegible], such as diabetic ret- inopa[illegible]retinop[illegible] of prematurity (ROP) and age-[illegible] mac[illegible] degeneration (AMD)[13-17]. [illegible]chola[illegible]rmed that IGF-1 gene acti- vati[illegible]lays an i[illegible]rtant role in the development of h[illegible]n myopia.

[illegible]urpose of this study was to inves- [illegible]e the changes of IGF-1/STAT3 pathway along [illegible] the expression of MMP-2 in sclera of the i[illegible]privation myopia guinea pigs. Our results coul[illegible]ovide a theoretical basis for further eluci- dating the molecular mechanism of myopia and provide a new molecular target for myo[illegible]

Materials and M[illegible]ds

Experimental Animals [illegible]d Grou[illegible]

A total of 24 three-w[illegible]old weanin[illegible] pigs, male or female [illegible]hout e[illegible] disease congenital myopia, [illegible]e coll[illegible]d. This work was approved by t[illegible]im[illegible]thics C[illegible]mit- tee of Affiliate[illegible]Hosp[illegible]Weifan[illegible]edical University A[illegible]al Cente[illegible]ine[illegible]gs were housed in a[illegible]erimental c[illegible]s with nat- ural circa[illegible]m and free a[illegible]ess to drink- ing wat[illegible]22-2[illegible] All guinea pigs were ran- domly divided into [illegible]s: group A, group B, gro[illegible]d group D. [illegible] A was considered a[illegible]ank control group without intervention. [illegible]mals were sa[illegible]ificed after feeding 21 days. [illegible]group B, the [illegible]nslucent mask was stitched [illegible]e right eye [illegible] 7 days. Next, members in g[illegible]B were [illegible]ificed. Guinea pigs in group C w[illegible]or 14 days. The eye patch was sewn to the right eye for 14 days before sac- rifice. In group D, the members were covered [illegible]s before sacrifice. In each group, the [illegible]ght [illegible] was used as an occluded eye, and the left eye as a self-control eye.

Animal Model Establishment and Data Collection

All the guinea pigs in treatment group were treated with 3% pentobarbital sodium intraperitoneal anesthesia followed by translucent eye mask fixed in the right eye. For diopter test, 0.15% tropicamide eye drops were used to paralyze ciliary muscle with dripping every five min for three times. After the pupil was fully dilated, refractive diopter was measured under streak retinoscopes. After anesthesia with ketamine, ocular surface anesthesia was performed with 1% tetracaine eye drops. Then the axial length was measured by A-mode ultrasonoscope.

Collecting Scleral Tissue

Diopter and axial length of guinea pigs were measured after covering the right eye for 7, 14, and 21 days, respectively. Guinea pigs were then sacrificed by cervical dislocation. The equatorial parts of the eyes were cut and then the eyeballs were cut off circularly to remove the anterior segment, vitreous body, retina and choroid, subsequently. Part of the sclera was fixed in 10% neu-

tral formaldehyde. The other part was stored in liquid nitrogen in the cryopreservation tube.

Hematoxylin-Eosin (HE) Staining in Scleral Tissue

The scleral specimens were fixed in neutral formalin for 24 h. Then the sections were routinely dehydrated, dipped in wax and embedded into four-micrometer scleral vertical sections. After HE staining, double-blind reading was performed under the optical microscope with 10 × 40 magnification.

Scleral MMP-2 and IGF-1 Expression Detected by Western Blot

Kidney tissues (0.1 g ± 0.05 g) were collected for protein extraction. The amount of sample was fixed. The primary antibodies (anti-STAT3 antibody, anti-MMP2 antibody, anti-IGF-1 antibody, Abcam, Cambridge, MA, USA) were added after conventional electrophoresis. Then the membrane bands were incubated overnight at 4°C. The next day the bands were incubated in the HRP(horseradish peroxidase) -labeled secondary antibody (Cell Signaling Technology, Danvers, MA, USA, anti-rabbit IgG, dilution: 1:5000) after being at room temperature for 2 h. The bands were developed by enhanced chemiluminescence (ECL) ing (Shanghai Biyuntian Biotechnology, Shang China). The integral optical density (IOD) value each band was measured with a g analysis system, with β-actin as an i al re ce. The relative expression of prote as calcu d by the ratio of IOD in each band tin IO

Statistical Analys

Statistical prod and service ns software package 1 Inc., Chicag , USA) were employed for the statistical analysis. Paired *t*-test was used in the data group alon way analysis of variance (ANOVA) g grou Results were expressed as mean tandard deviation. Least Significant Diff (LSD) was used as the post hoc test to ident ificance between groups. The relati hip bet e ex pressions of IGF-1 and M -2 were ana Pearson's correlation a sis $p<0.05$ was co ered as statistically s icant.

Resu

Compari iopter and ial Length of Eac oup

We compared diop d axial length of guinea p ng groups. T ults showed that the a ength and refraction of right eyes in groups , and D signi antly increased at the 7th day, d, and 21st mpared with the left eye of own and th ght eye of group A, respecti n<0.05) le I).

Eye Pathology of Groups of Guinea Pigs

In control group, the thickness of sclera of guinea normal. Collagen fibers were arranged atly, ormalized and uniform in diameter with extracellular matrix evenly distributed. The sclera of guinea pigs after special treatment for 7 and 14 days were significantly thinned, disordered, broken and separated with the collagen fibers distributed sparsely. And the diameter of which also decreased dramatically. In addition, we also found that gap between the fibers and extracellular matrix significantly increased. The guinea pigs in 21 days group showed more significant changes than those of the previous groups (Figure 1).

Table I. Co ison of diopter l length of each group (mean ± standard deviation).

G		7 d		14 d		21 d	
		Diopter ( )	Axis oculi (mm)	Diopter (mm)	Axis oculi (mm)	Diopter (mm)	Axis oculi (mm)
A	eye	55±1.10	7.65±0.25	2.51±0.09	7.85±0.06	2.56±0.09	7.86±0.08
		2.46±0.11	7.73±0.14	2.48±0.13	7.77±0.09	2.59±0.13	7.74±0.18
B	Righ	-1.39±0.14*#	8.19±0.07*#				
	Left eye	2.44±0.09	7.66±0.09				
C	ight eye	-1.63±0.11*#	8.15±0.07*#	-2.63±0.23*#	8.35±0.08*#		
		2.52±0.09	7.76±0.11	2.37±0.09	7.59±0.14		
	Right eye	-1.72±0.09*#	8.17±0.03*#	-2.41±0.09*#	8.41±0.09*#	-5.59±0.12*#	8.78±0.13*#
	Left eye	2.42±0.10	7.69±0.11	2.51±0.10	7.84±0.06	2.60±0.13	7.57±0.19

*: red with the control group A, the difference was statistically significant, p<0.05; #: Compared with their own left eye, t fference was statistically significant.

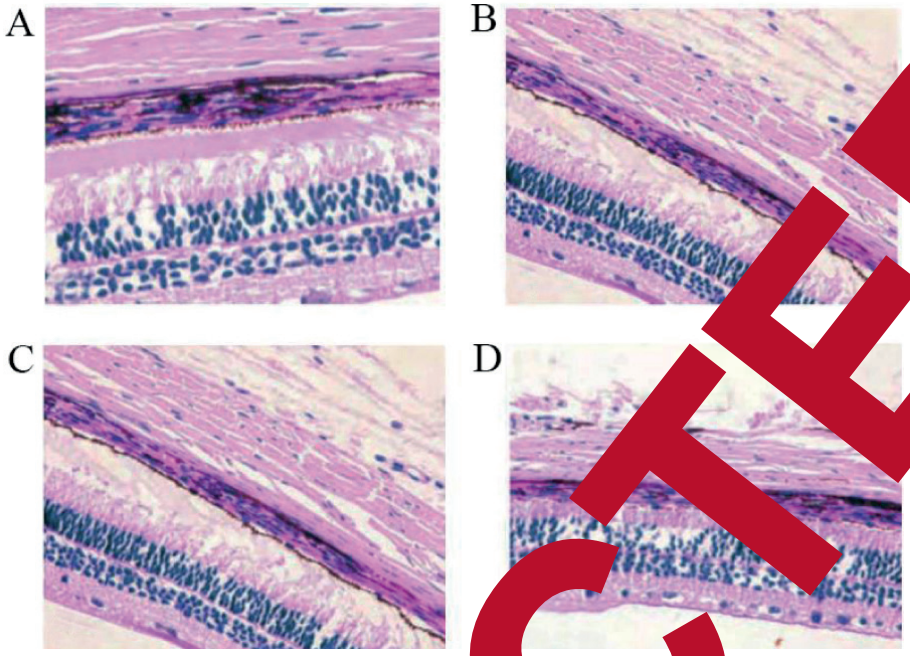
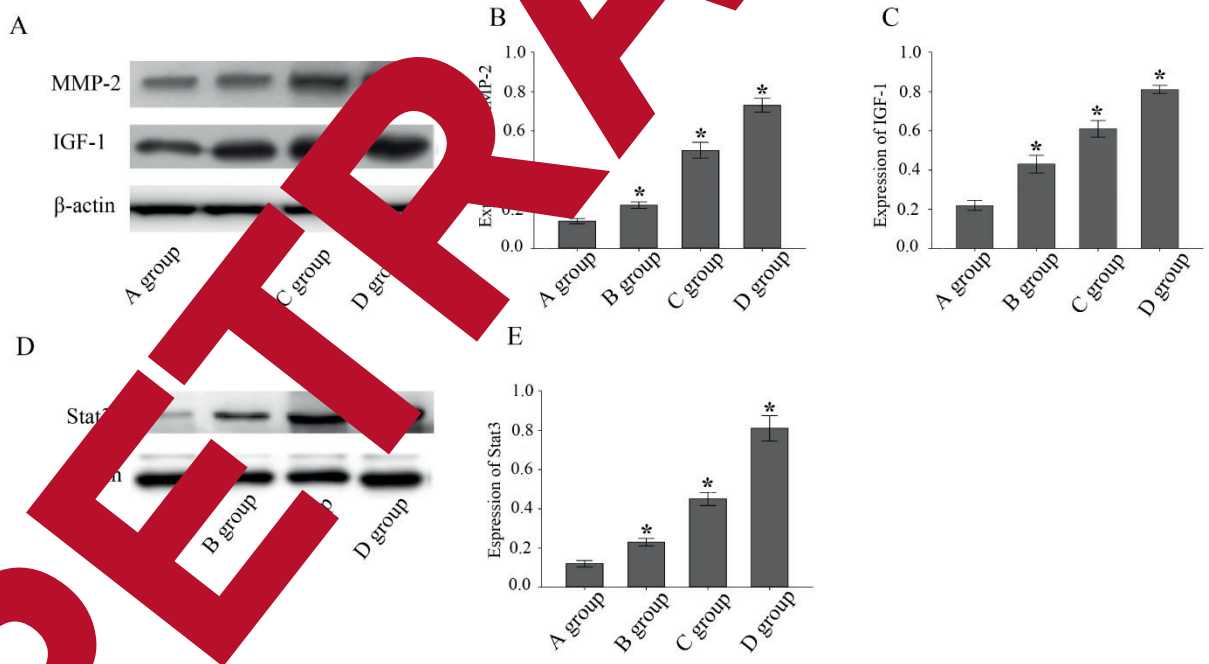


Figure 1. Histopathological changes in the covered eye of guin[illegible]s in each grou[illegible] In control group, the thickness of sclera of guinea pigs was normal, the collagen fibers were arranged[illegible] normaliz[illegible]iform in diameter and extracellular matrix little and equally distributed in. ***B-C***, The sclera of guinea pig[illegible] group were significantly thinned, the collagen fibers were distributed sparsely and disordered, broken and separa[illegible] sclera of guinea pig further was thinned after 21 days of masking with collagen fibers fractu[illegible]parated, the interspaces between fibers increased and the extracellular matrix increased (400×).



Fig[illegible]on of IGF-1, STAT3 and MMP-2 expressions in scleral tissue between guinea pigs in each group. ***A***, After 7[illegible] and 21[illegible] expression of MMP-2 in scleral tissue of guinea pig covered eyes (right eye) was significantly higher than [illegible]of the control group. ***B***, After 7^d, 14^d, and 21^d, the expression of IGF-1 in scleral tissue of guinea pigs covered eyes (right [illegible]as significantly higher than that of the control group. ***C***, After 7^d, 14^d, 21^d, the expression of STAT3 in scleral tissue of gu[illegible] covered eyes (right eye) was significantly higher than that of the control group. *: Compared with the control group A, th[illegible]erence was statistically significant, $p<0.05$.

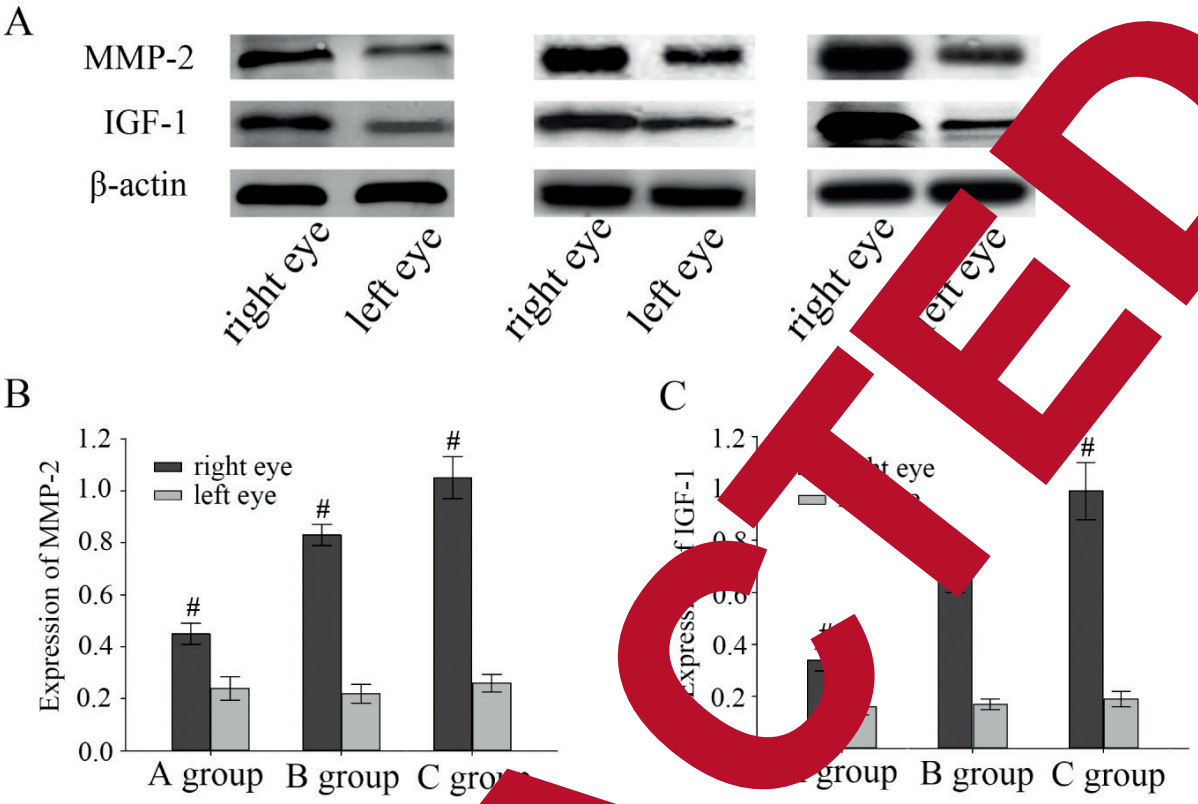


Figure 3. Comparison of IGF-1 and MMP-2 expre in s eyes of guinea pigs and that of their own left eye with the same cover time. ***A,*** In the same cover time, expres 2 in scleral of the covered eyes (right eyes) was significantly higher than that of the left eyes. ***B,*** IGF-1 the scleral tissue was significantly increased in the guinea pig covered (right eye) eyes compared with the left eye e cover time. #: Compared with the expression of sclera in left eye, the difference was statistically si ficant (p<0.0

MMP-2 and IGF-1 Exp ion Co arison in Sclera of Each Gr

Western blot results owe pressions of IGF-1, STAT3 and M 2 in the l group than those of treatment p. With the p ation of the occlusion ti sions of abov ee proteins in the tr ent g t the 7th d, 14th d, and 21st d incre ed gradually re 2). Meanwhile, we also o ved expression s of IGF-1 and MMP-2 ween the right eye a d the left eye of the s guinea n the experimental group. We fou t, the ression f IGF-1 and MMP-2 in the sc of the o ed eye were significantly higher t se in eft eye (p<0.05 Figure 3).

Co ation B een MMP-2 and IGF-2 Exp sion in Sclera of Guinea Pigs with F ion Myopia

orrelation analysis showed that the protein essions of MMP-2 and IGF-1 in scleral were s correlated (R=0.962, p<0.01) at different stag form deprivation. The above results indicated that both of them were closely associated with formation of deprivation myopia. These two proteins may jointly promote the increase of diopter and ocular axis elongation, eventually leading to myopia (Figure 4).

Discussion

In our study, the monocular form deprivation myopia models in guinea pigs were successfully established. With the prolongation of occlusion time, the degree of myopia of the occluded eyes increased. The differences of diopter and axial length between covered eyes and control eyes were statistically significant, indicating that form deprivation could give rise to axis extension. The myopia caused by form-deprivation is axial myopia, suggesting that we successfully established form deprivation myopia model in guinea pig.

The current researches have shown that the form deprivation regulates the growth of adja-

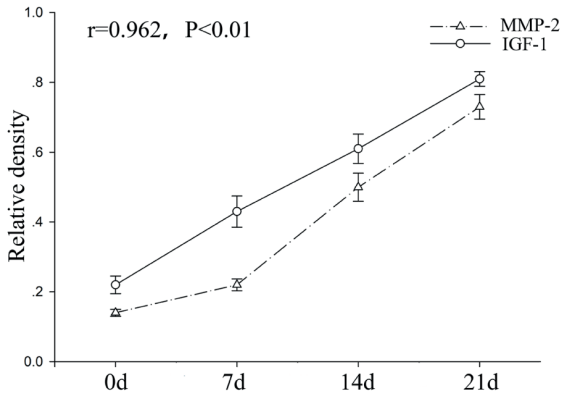


Figure 4. Correlation diagram of protein expressions of MMP-2 and IGF-1 in sclera at different occlusion time points. With the extension of time, the expression of MMP-2 and IGF-1 in the sclera of guinea pigs showed an upward trend with a strong correlation. $p<0.01$.

cent sclera mainly through the local retinal mechanism and can lead to the expression changes of many neurotransmitters and growth factors in the retina. As a first-class messenger, these neurotransmitters and growth factors act on the retinal pigment epithelium cells and cho[roid] to produce secondary messengers, thus a[ffect] the synthesis or degradation of the extrac[ellular] material (ECM). As a result, sclera is res[haped] and the ocular axis prolonged, thereafter lead[ing] to myopia at last[21-23]. Collagen in [the] mammal scle[ra] accounts for about 90% of scl[eral] [dry] weight, type I collagen accounts for most [of the] tota[l] [scle]ra collagen. Abnormal regulato[n of] sclera [cou]ld lead to dysfunctions of eye g[rowth] and process, eventually r[esulti]ng in incompatibility between axis grow[th] and re[fra]ctive condition, and even refracti[ve] errors[24,25].

STAT3, one [of] the members of the STAT's family, is involve[d] in a se[ries] of physiological changes such as cell proliferation, [dif]ferentiation and cell cycle[26,27]. STAT3 monomer i[n] the cytoplasm is inact[ive]. The activation of STAT3 mainly depend[s on] the JAK tyrosine kinase[28]. Therefore, tyr[osine] in STAT3 mol[ecu]le is phosphorylated by JA[K] to an act[ive] dimer. The dimer subsequently [tran]slocate[s] into the nucleus and binds DNA, fu[rther] acting to the STAT3 signal tran[sduc]tion. STAT3 mediates the signal transduct[ion] of many cytokines and growth factors to th[us] by affecting the transcription of target genes and regulates the function of cells. MMP-2 is one of the downstream target genes of STAT3 signal transduction pathway[29]. MMP-2 is an important gene that regulates sclera remodeling after myopia and plays an important role in the occurrence and development of my[opia]. The balance of MMP-2 expression play[s an] essential role in the extracellular matrix [me]tabolism of the sclera. Jones et al[31] conduct[ed a] study on the correlation between MMP-2 and [myopia] in 1996. They found that the activity of myofibroblast[s] and gelatinase A markedly increase[d] in the formation myopia. Rada et al also found that MMP-2 activity in the poste[rior] sclera in form-deprivation myopia model [was] significantly higher than that of control eyes.

IGF-1 is considered to participate various biological processes, including cell proliferation, differentiation, apoptosis, blood sugar maintenance and immune reaction regulation. Meanwhile, IGF-1 is widely expressed in eyes and involved in the development of various ophthalmic diseases[32-34]. Functionally, the mRNA expression of IGF-1R was detected in the posterior sclera of chicks. With the growth of the eyeball being drastically accelerated, mRNA expression of IGF-1R increased significantly in the posterior pole sclera of the covered eye with the occlusion time prolonging. The mRNA level of IGF-1 receptor in the sclera of the posterior pole of the eye was significantly higher than that of the control eyes after masking, whereas the level of IGF-1R began to decline after de-masking[35]. Penha et al[36] showed that IGF-1 injection into the glass of chicken can lead to diopter change and axial extension, resulting in the changes of the shape of the eye. In addition to animal experiments, Metlapally et al[37] also found a genetic relationship between high degree of myopia and IGF1.

Semi-quantitative analysis of Western blot showed that MMP-2 was expressed in scleral tissue of both experimental and control eye, while the expression level in control eye was relatively lower. With extension of treatment time, the expressions of MMP-2 and IGF-1 in the sclera of right eyes gradually increased on the 7th, 14th, and 21st day after masking, respectively. Above results suggested that MMP-2 and IGF-1 might be involved in the formation of form-deprivation myopia. Correlation analysis showed that the expressions of MMP-2 and IGF-1 in scleral tissue were strongly correlated ($r=0.962$, $p<0.01$) at different stages of deprivation, indicating that both of them are involved in the formation of FDM.

Based on those results, we suggested that there is an interaction between MMP-2 and IGF-1 during the development of form-deprived myopia. Kenney et al[38] found that inhibiting the IGF-1

pathway down-regulates the expression of MMP-2 in the sclera of guinea pigs and reduces the remodeling of the sclera. Therefore, IGF-1 may be an upstream regulating molecule of MMP-2. Evidence also demonstrated that overexpression of MMP-2 and IGF-1 during myopia formation might be responsible for scleral remodeling. However, the specific signaling pathway that regulates scleral remodeling is still not fully elucidated. The mechanism of MMP-2 in the formation and development of myopia remains to be further studied. It has been found that various biological factors can regulate MMP-2 expression. Further researches will be needed to clarify the pathogenesis of myopia and to develop new highly selective MMP-2 inhibitors.

Conclusions

We observed that form-deprivation of guinea pigs can enhance the expressions of GF-1, STAT3 and MMP-2 in the sclera and cause myopia in guinea pigs. The expressions of IGF-1, STAT3 and MMP-2 increased progressively with pro[illegible] time of deprivation. Additionally, overex[illegible] of MMP-2 mediated by IGF-1/STAT3 path[illegible] in sclera may promote the formation of myopia.

Conflict of Interest

The Authors declare that they hav[illegible] conflic[illegible] interest.

Ref[illegible]nc

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