

Original Research

Effects of latanoprost on the expression of TGF- β 1 and Wnt / β -catenin signaling pathway in the choroid of form-deprivation myopia rats

Nan-Cuo Suo¹, Chun-ling Lei², Yan-Chun Zhang², Xin-zhang Li¹, Feng-Zhi Li², Ke Gong^{2*}¹Department of Ophthalmology, Qinghai Red Cross Hospital, Xining, Qinghai, 821000, China²Department of Ophthalmology, Xi'an No. 4 Hospital, Xi'an, Shaanxi, 710004, China*Correspondence to: gongke4452@163.com

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Abstract: In this study, we investigated the effect of latanoprost on the expression of TGF- β 1 and Wnt / β -Catenin signal pathway in the choroid of form-deprivation myopia model rats. Forty rats were randomly divided into two groups: the control group and the FDM model group. Each group had 20 rats. The FDM model group was established by feeding latanoprost daily for 28 days. 15 rats in each group were used to measure the length of the ocular axis and the level of TGF- β 1 in choroidal tissue; the remaining 5 rats in each group were used for choroidal fibroblast culture. After modeling, the rats were killed, the length of the ocular axis was measured with a vernier caliper, and the level of TGF- β 1 protein and mRNA in the choroidal tissue of each group were measured with RT-PCR method. Results showed that compared with the control group, there was a significant difference in the axial length of the FDM model group ($P < 0.05$). There was a significant difference in the expression of TGF- β 1 protein and mRNA between the two groups ($P < 0.05$). The cultured cells were identified as choroidal fibroblasts by immunocytochemistry. There was no significant difference ($P > 0.05$) in the comparison of GSK3 β protein in choroidal fibroblasts of rats in each group. TGF- β 1 and APC protein in FDM group were significantly lower than those in the control group ($P < 0.05$), while dcl3, p21-gsk3 β and β -Catenin proteins were significantly higher ($P < 0.05$), and there was no significant difference ($P > 0.05$) in the ratio of various indexes protein in FDM + ddk1 group and the comparison of TGF- β 1 and APC protein in FDM + ddk1 group and FDM group. The expression of dcl3, p21-gsk3 β and β -Catenin decreased significantly ($P < 0.05$). There was no significant difference in the expression of GSK3 β mRNA in the choroidal fibroblasts of each group ($P > 0.05$). The expression of TGF- β 1 and APC mRNA in FDM group was significantly lower than that in the control group ($P < 0.05$), while the expression of dcl3, p21-gsk3 β and β -catenin mRNA in FDM + ddk1 group was significantly higher than that in the control group ($P < 0.05$). In FDM + ddk1 group, TGF- β 1 and APC mRNA were significantly lower than those in FDM group ($P < 0.05$), while dcl3, p21-gsk3 β and β -Catenin mRNA were significantly higher ($P < 0.05$).

Key words: Latanoprost; Choroid; Form-deprivation myopia; TGF- β 1; Wnt / β -catenin signaling pathway.

Introduction

Myopia caused by refractive errors in the eye that many people can be born with or age with. This problem is one of the most common eye refractive errors and has become more common in recent years. Myopia is not a disease; it is a complication. Unlike far-sighted people who have smaller eyeballs, people with myopia typically have larger eyeballs. These people are clearly able to see near objects, while objects appear farther away. This problem can be corrected with glasses, contact lenses or surgery. There is a high incidence of myopia in the world, especially in Asia. High myopia can cause serious complications, which is a common cause of blindness in adults. However, there is still no understanding of its pathogenesis, and there is no effective treatment (1). Therefore, exploring the pathogenesis of myopia can provide a theoretical basis for the treatment of myopia. Wnt / β -catenin signaling pathway plays an important role in regulating a variety of life activities. According to research, Wnt / β -catenin signaling pathway is closely related to the occurrence and development of myopia, and it can participate in the process of myopic occurrence and development such as apop-

toxis, inflammatory response and angiogenic regulation after activation (2). TGF- β 1 (transforming growth factor- β 1) is expressed in sclera, retina and choroid (3), the research shows that the expression level of TGF- β 1 in the choroid of myopic rats is lower than that of normal rats (4), but the correlation between TGF- β 1 and Wnt / β -Catenin signal pathway is not clear at present, so the purpose of this paper is to explore the effect of latanoprost on the model of form-deprivation myopia. The expression of TGF- β 1 and the regulation of Wnt / β -catenin signaling pathway in the choroid of rats provide a new basis for the treatment of myopia.

Materials and Methods

Experimental animal

40 3-week-old SD rats, body weight 66-72g, male and female, purchased from Shanghai slake experimental animal Co., Ltd., animal license No.: scxk (Lu) 2007-0005.

Main reagents and instruments

RT-PCR kit, fetal bovine serum, Trizol, high glucose DMEM medium, BCA kit and Dickkopf related protein

1 (ddk1), Wnt / β - Catenin signal pathway inhibitor (Invitrogen, USA), β - actin antibody, Rabbit anti-mouse TGF- β 1 polyclonal antibody, Rabbit anti-mouse type 3 glyconuclease (dcl-3) polyclonal antibody, Rabbit anti-mouse type 3 colon adenoma like information The meat protein (APC) polyclonal antibody, Rabbit anti-mouse glycogen synthetase hormone 3 β (GSK3 β) polyclonal antibody, Rabbit anti-mouse p21-gsk3 β polyclonal antibody and Rabbit anti-mouse β - Catenin polyclonal antibody (sigma company of the United States), TGF- β 1, dcl3, APC, GSK3 β , p21-gsk3 β and β - Catenin introductions were designed and synthesized by Shanghai Bioengineering Technology Co., Ltd. Bio-Rad PCR instrument (bio company in America), Olympus BX41 digital microscope (Olympus company in Japan).

Experimental animal grouping and model establishment

40 rats were randomly divided into the control group and FDM model group, 20 rats in each group. FDM model group rats established FDM model: rats were fed with latanoprost every day for 28 days, 15 rats in each group were used to measure the length of ocular axis and the level of TGF- β 1 in choroidal tissue; the remaining 5 rats in each group were used for choroidal fibroblast culture.

Measurement of the axial length of the eye in each group

At the end of modeling, the rats were killed by excessive anesthesia, the right eyeball was extracted, and the length of the ocular axis was measured by vernier caliper.

Detection of TGF - β 1 protein and mRNA in choroidal tissue of rats in each group

After measuring the length of the ocular axis of the rat, the choroidal tissue of the rat was separated, and the total protein of the choroidal tissue was extracted. The expression level of TGF- β 1 protein in the choroid was measured by the BCA method. The Rabbit anti-rat TGF- β 1 polyclonal antibody was used as an antibody, and β - actin was used as an internal reference. The relative expression level of TGF- β 1 protein = the gray value of TGF - β 1 protein strip / the gray value of the β - actin strip. The choroidal tissue of rats was completely ground in the grinder, and the total RNA of choroidal tissue was extracted. The TGF- β 1 mRNA in the choroidal tissue of rats in each group was measured by RT-PCR. The PCR reaction conditions were: 95 °C, 30s; 95 °C, 15s; 60 °C, 30s; 95 °C, 15s, 40 cycles in total; 60 °C, 1min; 95 °C, 15s. The relative expression level of TGF- β 1 mRNA in the choroid tissue of rats was calculated by the $2^{-\Delta\Delta Ct}$ method.

Culture and identification of rat choroidal fibroblasts

The remaining 5 rats in each group were killed, choroidal tissues were taken out, cut into pieces, inoculated into culture bottles, incubated in EMEM medium, changed the solution once in three days, after the fusion of more than 85% cells, digested with trypsin, and sub-cultured. The growth of choroidal cells was observed under an inverted microscope every day. The third gen-

eration of cells in good growth condition was used to prepare climbing tablets, which were identified by immunofluorescence cytochemistry: after climbing tablets, the cells were fixed with polyformaldehyde for 15 minutes, broken the membrane for 30 minutes, sealed with serum for 30 minutes, incubated overnight with vimentin monoclonal antibody, incubated for 2 hours with goat anti-rabbit IgG FITC, incubated for 10 minutes with DAPI working solution, sealed and observed the staining.

Grouping and treatment of choroidal fibroblasts

The choroidal fibroblasts in the control group were used as the control group. The choroidal fibroblasts in the FDM model group were divided into two groups: FDM group and FDM + ddk1 group. The third generation choroidal fibroblasts with good growth were inoculated into 96 well plates (the cell density was 5×10^3 cells / well), each group was set with 8 compound holes. After 24 hours of DMEM culture, the cells grew on the wall Ddk1 ($10 \mu\text{g} \cdot \text{L}^{-1}$) was added to the choroidal fibroblasts of the control group and FDM group, and the same amount of culture medium was added to the choroidal fibroblasts of the control group and FDM group.

Expression of TGF - β 1, dcl3, APC, GSK3 β , p21-gsk3 β and β - Catenin protein and mRNA in choroidal fibroblasts of rats in each group

The expression of TGF - β 1, dcl3, APC, GSK3 β , p21-gsk3 β and β - Catenin in the choroidal fibroblasts was measured by western blotting. The first antibody was Rabbit anti-mouse TGF- β 1 polyclonal antibody, Rabbit anti-mouse dcl3 polyclonal antibody, Rabbit anti-mouse APC polyclonal antibody, Rabbit anti-mouse GSK3 β polyclonal antibody and Rabbit anti-mouse p21-gsk3 β polyclonal antibody. The antibody and Rabbit anti-mouse β - Catenin polyclonal antibody are one antibody. The expression of TGF- β 1, dcl3, APC, GSK3 β , p21-gsk3 β and β - Catenin protein and mRNA were measured by RT-PCR.

Statistical treatment

SPSS 20.0 statistical software was used for statistical analysis. The length of the ocular axis, the expression of TGF- β 1 protein and mRNA in the choroid tissue of rats. The expression levels of TGF- β 1, dcl3, APC, GSK3 β , p21-gsk3 β and β - Catenin protein and mRNA in choroidal fibroblasts of rats were expressed by ($\bar{x} \pm s$), and the homogeneity of variance was tested by F test. T-test was used for comparison between the two groups, the single-factor analysis was used for comparison between multiple groups, SNK-q test was used for comparison between the two components, $\alpha = 0.05$ as the test level.

Results

Comparison of the length of the ocular axis between the two groups

Compared with the control group [(7.37 ± 0.13) mm], the length of the eye axis in the FDM model group increased significantly ($P < 0.05$).

Comparison of the expression of TGF - β 1 protein and mRNA in choroidal tissue between two groups of rats

See Table 1 and Figure 1. There was a significant difference in the expression of TGF-β1 protein and mRNA between the two groups ($P < 0.05$).

Culture and identification of rat choroidal fibroblasts

See Figure 2. In the primary culture, cells crawled out from the edge of the tissue block for three days, showing a long fusiform shape and transparent cytoplasm; after 12 days of culture, cells fused into monolayer cells. The cells began to adhere to the wall for 24 hours and became fusiform. After 3 days, the cells fused. After immunocytochemical identification, more than 95% of the cultured cells expressed vimentin, indicating that the cultured cells were choroidal fibroblasts.

Table 1. Comparison of the expression levels of TGF - β1 protein and mRNA in choroidal tissues between the two groups ($\bar{x} \pm S$).

Group	TGF -β1 protein	TGF-β1mRNA
Control	0.61±0.13	1.00±0.02
FDM model	0.27±0.06	0.57±0.13
P	<0.05	<0.05

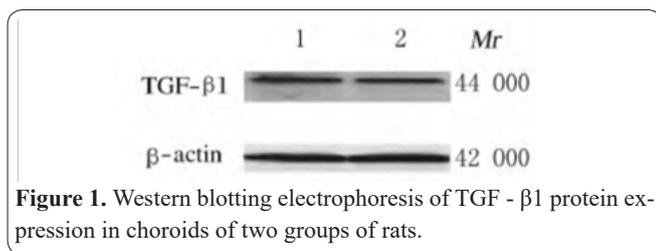


Figure 1. Western blotting electrophoresis of TGF - β1 protein expression in choroids of two groups of rats.

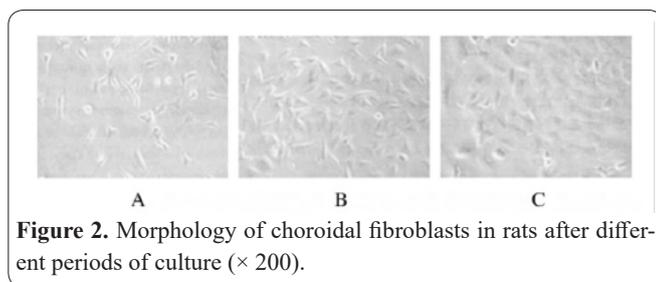


Figure 2. Morphology of choroidal fibroblasts in rats after different periods of culture ($\times 200$).

Table 3. Comparison of TGF - β 1, dcl3, APC, GSK3 β, p21-gsk3 β and β - Catenin mRNA expression levels in choroidal fibroblasts of rats in each group ($\bar{x} \pm S$).

Group	TGF-β 1mRNA	DCL3 mRNA	APC mRNA	GSK3β mRNA	p21-GSK3β mRNA	β-catenin mRNA
Control	1.01±0.04	1.01±0.03	1.02±0.02	1.02±0.03	1.02±0.04	1.03±0.02
FDM	0.43±0.08	2.32±0.43	0.52±0.14	0.98±0.016	2.54±0.37	1.96±0.35
FDM+DDK1	0.97±0.15	1.08±0.22	0.97±0.18	0.98±0.114	1.08±0.18	1.07±0.24
P	<0.05	<0.05	<0.05	P>0.05	<0.05	<0.05

Expression of TGF - β 1, dcl3, APC, GSK3 β, p21-gsk3 β and β - Catenin in choroidal fibroblasts of rats in each group

As shown in Table 2 and Figure 3, there was no significant difference ($P > 0.05$) in the comparison of GSK3 β protein in choroidal fibroblasts of rats in each group. The TGF - β 1 and APC protein in choroidal fibroblasts of rats in FDM group were significantly lower than those in the control group ($P < 0.05$), while those in dcl3, p21-gsk3 β and β - Catenin were significantly higher ($P < 0.05$), while those in choroidal fibroblasts of rats in FDM + ddk1 group were not. There was a significant difference ($P > 0.05$). The expression of TGF - β 1 and APC protein in the FDM + ddk1 group was significantly higher than that in the FDM group ($P < 0.05$), while the expression of dcl3, p21-gsk3 β and β - Catenin protein was significantly lower ($P < 0.05$).

Expression of TGF - β 1, dcl3, APC, GSK3 β, p21-gsk3 β and β - Catenin mRNA in choroidal fibroblasts of rats in each group

As shown in Table 3, there was no significant difference ($P > 0.05$) in the expression of GSK3 β mRNA in

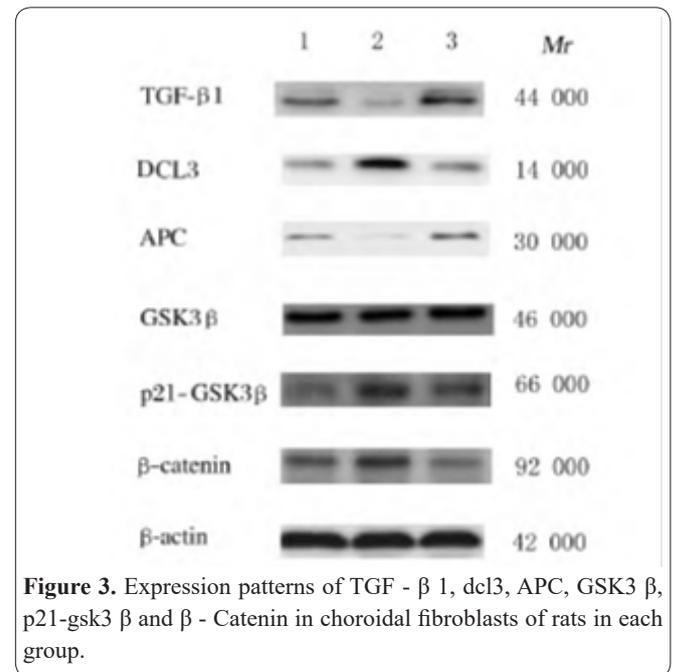


Figure 3. Expression patterns of TGF - β 1, dcl3, APC, GSK3 β, p21-gsk3 β and β - Catenin in choroidal fibroblasts of rats in each group.

Table 2. Comparison of TGF - β 1, dcl3, APC, GSK3 β, p21-gsk3 β and β - Catenin protein expression levels in choroidal fibroblasts of rats in each group ($\bar{x} \pm S$).

Group	TGF-β1 protein	DCL3 protein	APC protein	GSK3β protein	p21-GSK3β protein	β-catenin protein
Control	0.59±0.15	0.24±0.06	0.62±0.13	0.42±0.07	0.32±0.08	0.38±0.08
FDM	0.27±0.09	0.62±0.14	0.36±0.08	0.44±0.04	0.73±0.16	0.84±0.17
FDM+DDK1	0.55±0.13	0.31±0.08	0.58±0.12	0.42±0.08	0.36±0.15	0.43±0.12
P	<0.05	<0.05	<0.05	P>0.05	<0.05	<0.05

the choroidal fibroblasts of each group. The expression of TGF - β 1 and APC mRNA in FDM group was significantly lower than that in the control group ($P < 0.05$), while the expression of dcl3, p21-gsk3 β and β - Catenin mRNA was significantly higher than that in the control group ($P < 0.05$). There was no significant difference in the expression of various indexes mRNA in the choroidal fibroblasts of FDM + ddk1 group. The expression of TGF - β 1 and APC mRNA in FDM + ddk1 group was significantly lower than that in FDM group ($P < 0.05$), while dcl3, p21-gsk3 β and β - Catenin mRNAs were significantly higher ($P < 0.05$).

Discussion

Myopia is a common refractive error. Under normal conditions, the axial length of the eye and the refractive system cooperate with each other, so that the parallel light from the external object can focus on the macular center of the retina through the refractive system to form a clear image. When the axial length of the eye increases, the parallel light from the external object can be imaged before the retina through the refractive system, thus forming myopia (5). With the onset of myopia with the increase in the rate, there are more and more serious complications caused by high myopia. The main complications of high myopia are scleral staphyloma, retinal hemorrhage, hole, atrophic degeneration, subretinal neovascularization, retinal detachment, glaucoma and cataract, which seriously affect human health (6). TGF - β 1 plays an important role in embryo development, cell differentiation, immune response and tissue regeneration Function (7,8), TGF - β 1 is one of the TGF - β subtypes, which is expressed in sclera, choroid and retina. TGF - β 1 is mainly distributed in ganglion cells, amacrine cells and bipolar cells. It participates in the regulation of choroidal remodeling by regulating collagen production and fibroblast proliferation, choroidal fibroblast and chondrocyte proliferation in FDM. The expression level of TGF - β 1 in choroidal tissue of rats decreased (8,9). The FDM rat model established in this paper found that the length of the ocular axis increased and the expression level of TGF - β 1 in the choroidal tissue of rats in the FDM model group decreased. The reason for the stage may be that TGF - β 1 participated in the formation of FDM, and further separated the choroidal fibroblasts (10), the choroidal fibroblasts isolated and cultured in FDM model group were fine. The expression level of TGF - β 1 in FDM rat choroidal fibroblasts was lower than that in the control group.

Wnt / β - catenin signaling pathway is a classic Wnt signaling pathway, which participates in the physiological and pathological processes such as inflammation, fibrosis and angiogenesis, and regulates the process of cell differentiation, proliferation, apoptosis and migration (11,12), including Wnt, β - Catenin, APC, GSK3 β , dcl3 and p21-gsk3 β . When Wnt / β - catenin signaling pathway is inactivated, β - Catenin in cytoplasm adheres to the cytoskeleton and mediates the intercellular. When Wnt / β - catenin signaling pathway is activated, β - Catenin enters into the cell nucleus to collect other activators and promote the expression of its downstream target genes (12,13). In the process, Wnt / β - catenin signaling pathway is activated, and it is involved in

myopia and its occurrence through biological functions such as neovascularization, apoptosis and extracellular matrix metabolism (14). The results of this study on Wnt / β - catenin signaling pathway in FDM rat choroidal fibroblasts show that β - Catenin negative regulatory protein APC in FDM rat choroidal fibroblasts. The level of dcl3 and p21-gsk3 β decreased, and the level of β - Catenin also increased (15), which indicated that the activity of Wnt / β - catenin signaling pathway was inhibited and the level of TGF - β 1 was increased after ddk1, which indicated that the Wnt / β - catenin signaling pathway might be involved in myopia by regulating the expression level of TGF - β 1. The process of occurrence. Evaluating the expression of key genes and controlling their expression are a way to prevent harmful traits and phenotypes (16-24). Genome editing may be effective in this regard (25). Gene regulation refers to the mechanisms by which cells perform a gene (protein or RNA) to increase or decrease a specific product. Every step from gene expression can be adjusted from the beginning of interpretation to the processing of the RNA and the modification of the protein from the translation of the RNA. Often in a gene regulation network, the regulator of different genes controls each other (26-27). In this research, the expression of TGF- β 1 and Wnt / β -catenin signaling pathway in the choroid of form-deprivation myopia rats was investigated.

Wnt / β - catenin signaling pathway may be involved in myopia by regulating the expression level of TGF - β 1. Inhibiting Wnt / β - catenin signaling pathway can increase the level of TGF - β 1, which plays an important role in controlling myopia.

References

- Li M, Yuan Y, Chen Q, Me R, Gu Q, Yu Y, Sheng M, Ke B. Expression of Wnt/ β -catenin signaling pathway and its regulatory role in type I collagen with TGF- β 1 in scleral fibroblasts from an experimentally induced myopia guinea pig model. *J Ophthalmol* 2016.
- Peiffer RL, Lee C, Geller AM, Tripoli NK, Maguire R, Rahman AA, Abou-Donia MB, Boyles WK. Dose-Effect for Inhibition of form-deprivation Myopia (FDM) by Chlorpyrifos (CPF); Roles of Retinal TGF- β 3 and Corneal Curvature. *J Toxicol Cutaneous Ocul Toxicol* 1999; 18(3):262-3.
- Hsieh YH, Hsieh MF, Fang CH, Jiang CP, Lin B, Lee HM. Osteochondral regeneration induced by TGF- β loaded photo cross-linked hyaluronic acid hydrogel infiltrated in fused deposition-manufactured composite scaffold of hydroxyapatite and poly (ethylene glycol)-block-poly (ϵ -caprolactone). *Polym* 2017; 9(5):182.
- de María Guillén-Jiménez F, Netzahuatl-Muñoz AR, Morales-Barreira L, Cristiani-Urbina E. Hexavalent chromium removal by *Candida* sp. in a concentric draft-tube airlift bioreactor. *Water Air Soil Pollut* 2009; 204(1-4):43.
- Mo Y, Wang Y, Cao B, Zhang J, Ren G, Yang T. Scleral TGF- β 1 and Smad3 expression is altered by TCM BU jing Yi SHI tablets in guinea pigs with form-deprivation myopia. *J Tradit Chin Med Sci* 2016; 3(2):124-32.
- Noh YK, Du P, Da Costa AD, Park K. Induction of chondrogenesis of human placenta-derived mesenchymal stem cells via heparin-grafted human fibroblast derived matrix. *Biomater Res* 2018; 22(1):12.
- Peiffer RL, Lee C, Geller AM, Tripoli NK, Maguire R, Rahman AA, Abou-Donia MB, Boyles WK. Dose-Effect for Inhibition of form-deprivation Myopia (FDM) by Chlorpyrifos (CPF); Roles of Retinal TGF- β 3 and Corneal Curvature. *J Toxicol Cutaneous Ocul Toxicol* 1999; 18(3):262-3.

8. Dravid G, Ye Z, Hammond H, Chen G, Pyle A, Donovan P, Yu X, Cheng L. Defining the role of Wnt/ β -catenin signaling in the survival, proliferation, and self-renewal of human embryonic stem cells. *Stem Cells* 2005; 23(10):1489-501.
9. Geller AM, Abdel-Rahman AA, Peiffer RL, Abou-Donia MB, Boyes WK. The organophosphate pesticide chlorpyrifos affects form deprivation myopia. *Invest Ophthalmol Vis Sci* 1998; 39(7):1290-4.
10. Kanzaki T, Olofsson A, Morén A, Wernstedt C, Hellman U, Miyazono K, Claesson-Welsh L, Heldin CH. TGF- β 1 binding protein: a component of the large latent complex of TGF- β 1 with multiple repeat sequences. *Cell* 1990; 61(6):1051-61.
11. King C, Davies J, Mueller R, Lee MS, Krahl T, Yeung B, O'Connor E, Sarvetnick N. TGF- β 1 alters APC preference, polarizing islet antigen responses toward a Th2 phenotype. *Immun* 1998; 8(5):601-13.
12. Han G, Lu SL, Li AG, He W, Corless CL, Kulesz-Martin M, Wang XJ. Distinct mechanisms of TGF- β 1-mediated epithelial-to-mesenchymal transition and metastasis during skin carcinogenesis. *J Clin Invest* 2005; 115(7):1714-23.
13. Bedossa P, Peltier E, Terris B, Franco D, Poynard T. Transforming growth factor-beta 1 (TGF- β 1) and TGF- β 1 receptors in normal, cirrhotic, and neoplastic human livers. *Hepatology* 1995; 21(3):760-6.
14. Björklund P, Åkerström G, Westin G. An LRP5 receptor with internal deletion in hyperparathyroid tumors with implications for deregulated WNT/ β -catenin signaling. *PLoS Med* 2007; 4(11).
15. Huang H, He X. Wnt/ β -catenin signaling: new (and old) players and new insights. *Curr Opin Cell Biol* 2008; 20(2):119-25.
16. Ghorbani T, Kahrizi D, Saeidi M, Arji I. Effect of sucrose concentrations on *Stevia rebaudiana* Bertoni tissue culture and gene expression. *Cell Mol Biol*, 63(11): 32-36.
17. Akbari F, Arminian A, Kahrizi D, Fazeli A, Ghaheri M. Effect of nitrogen sources on gene expression of *Stevia rebaudiana* (Bertoni) under in vitro conditions. *Cell Mol Biol*, 64(2): 11-16.
18. Akbarabadi A, Ismaili A, Kahrizi D, Firouzabadi FN. Validation of expression stability of reference genes in response to herbicide stress in wild oat (*Avena ludoviciana*). *Cell Mol Biol* 2018; 64(4): 113-118.
19. Ghaheri M, Adibrad E, Safavi SM, Kahrizi D, Soroush A, Mohammadi S, Ghorbani T, Sabzevari A, Ansarypour Z, Rahmanian E. Effects of life cycle and leaves location on gene expression and glycoside biosynthesis pathway in *Stevia rebaudiana* Bertoni. *Cell Mol Biol*, 64(2): 17-22.
20. Kahrizi D, Ghaheri M, Yari Z, Yari K, Bahraminejad S. Investigation of different concentrations of MS media effects on gene expression and steviol glycosides accumulation in *Stevia rebaudiana* Bertoni. *Cell Mol Biol*, 64(2): 23-27.
21. Hashempour S, Ghaheri M, Kahrizi D, Kazemi N, Mohammadi S, Safavi SM, Ghorbani T, Rahmanian E, Heshmatpanaah M. Effects of different concentrations of mannitol on gene expression in *Stevia rebaudiana* Bertoni. *Cell Mol Biol*, 64(2): 28-31.
22. Kahrizi D, Ghari SM, Ghaheri M, Fallah F, Ghorbani T, Beheshti AA, Kazemi E, Ansarypour Z. Effect of KH₂PO₄ on gene expression, morphological and biochemical characteristics of *Stevia rebaudiana* Bertoni under in vitro conditions. *Cell Mol Biol*, 63(7): 107-111.
23. Esmaeili F, Ghaheri M, Kahrizi D, Mansouri M, Safavi SM, Ghorbani T, Mohammadi S, Rahmanian E, Vaziri S. Effects of various glutamine concentrations on gene expression and steviol glycosides accumulation in *Stevia rebaudiana* Bertoni. *Cell Mol Biol*, 64(2): 1-5.
24. Fallah F, Nokhasi F, Ghaheri M, Kahrizi D, Beheshti AA, Ghorbani T, Kazemi E, Ansarypour Z. Effect of salinity on gene expression, morphological and biochemical characteristics of *Stevia rebaudiana* Bertoni under in vitro conditions. *Cell Mol Biol* 2017; 63(7): 102-106.
25. Bordbar M, Darvishzadeh R, Pazhouhandeh M, Kahrizi D. An overview of genome editing methods based on endonucleases. *Mod Genet J* 2020; 15(2): 75-92.
26. Ghaheri M, Kahrizi D, Bahrami G, Mohammadi-Motlagh HR. Study of gene expression and steviol glycosides accumulation in *Stevia rebaudiana* Bertoni under various mannitol concentrations. *Mol Biol Rep* 2019; 46(1): 7-16.
27. Eruygur N, Ucar E, Akpulat HA, Shamsavari K, Safavi SM, Kahrizi D. In vitro antioxidant assessment, screening of enzyme inhibitory activities of methanol and water extracts and gene expression in *Hypericum lydium*. *Mol Biol Rep* 2019; 46(2): 2121-9.