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RESEARCH ARTICLE

Stem cell-derived nano-scale vesicles promotes the proliferation of retinal ganglion cells (RGCs) by activation PI3K/Akt and ERK pathway

Hamed Mahmoudi[1], Alireza Nouralishahi[2,3], Saman Mohammadzadehsaliani[4*]

[1] Resident of Oral &Maxillofacial Surgery, School of Dentistry, Tehran University of Medical Science, Tehran, Iran.

[2] Isfahan Eye Research Center, Isfahan University of Medical sciences, Iran

[3] KIMS Health Hospital, Oman

[4] Department of Ophthalmology, Rustaq Hospital, Rustaq, Oman

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ABSTRACT

Objective(s): To evaluate the effects of the mesenchymal stem cell-derived exosome on the proliferation of retinal ganglion cells (RGCs)-5 cell line.

Methods: Exosomes were isolated from the human bone marrow (BM)-derived MSCs. Exosomes characteristic were verified by western blotting and transmission electron microscopy (TEM) image. The proliferation of the RGC-5 cell line was estimated in co-culture conditions with MSCs-exosome by MTT assay within 24-96 hours of exposure. The expression levels of the AKT, PI3K, and ERK pathways were measured in RGC-5 cells within 24-96 hours of exposure.

Results: Based on the MTT assay results, exosomes at concentrations of 50-200 ng/ml enhanced the proliferation of the RGC-5 cell line in vitro. Also, this co-culture resulted in the up-regulating of the expression of AKT, PI3K, and ERK pathways in the RGC-5 cell line.

Conclusions: Accordingly, we concluded that the exosome could stimulate the proliferation of the RGC-5 cell line by inducing PI3K/AKT and ERK axes.

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INTRODUCTION

Retinal ganglion cells (RGCs), as the main type of neuron situated neighboring the inner surface of the retina, make interactions between the retinal input as well as the visual processing centers in CNS [1, 2]. There exist a notable variety of RGCs, and each of them possesses exclusive morphological properties, different activity, and characteristic [3]. These cells, as described, link the inner retina to the responding brain segment. A diversity of psychophysical and electrophysiological examinations have been evolved and conducted to assess the large and diverse residents of RGCs [4, 5]. The RGCs loss is the influential parameter in various ocular disorders, such as optic nerve injury (ONI), retinal ischemia, and also glaucoma [6, 7]. Such conditions bring about impairment in vision loss and blindness in some cases. For instance, glaucoma, the second main reason for blindness worldwide, has been shown to be mainly revealed in ganglion cells [8]. Thus, improving the proliferation and regeneration of RGCs is of paramount importance.

Current studies designated that pleiotropic impacts of mesenchymal stem cells (MSCs) are mainly induced by secreting soluble paracrine biomolecules, including cytokines, growth factors, and nucleic acid [9, 10]. Exosomes, as the chief kinds of nano-scale extracellular vesicles (EVs),

* Corresponding Author Email: *farhad20091367@gmail.com*

are 30-200 nm in diameter. The EVs are typically classified into three main sub-populations. Apart from exosomes, apoptotic bodies (ApoB) and microvesicles (MVs), which both of them are larger than 100 nm in diameter, are other subtypes [11]. Exosomes typically are generated by growing as intraluminal vesicles (ILVs) through the luminal space of the late endosomes [12, 13]. Exosomes transport pivotal cargos like miRNA and mRNA, proteins, and lipids given from parental cells to the recipient cells. They ease damaged tissue regeneration and support the healing of destructed tissues as well as organs [14, 15]. The central roles of MSCs-derived exosomes in the stem cell-mediated therapeutic impacts have strongly been ascertained in numerous experimental models. Notably, exosomes elicit comparable influences to MSCs, more efficiently promoting tissue repair, inducing new vessel generation, regulating immune responses, and finally, neuroprotection [16, 17]. In fact, they enable such effects by transporting the biological data between cells in physiological and also pathological status [18].

In this study, we have evaluated the possible effects of the MSCs-derived exosome on the RGC-5 cell line in vitro.

MATERIALS AND METHODS

Cell culture

The human MSCs (Royesh Stem Cell Biotechnology, Tehran, Iran) were cultivated in DMEM with 10 percent FBS (Sigma-Aldrich, Germany). Then, cells were kept at a 37°C humidified atmosphere, including the CO2 5%. Also, RGC-5 cells (ATCC, USA) were cultivated in 1 mg/mL glucose DMEM, 10% FBS and 1% pen/strep.

Exosome isolation

Stem cell-derived exosomes were acquired from the serum-free conditioned media (CM) using the MagCapture™ Exosome Isolation Kit based on manufacturer instruction. For this purpose, the CM was centrifuged at 10000 × g for 20 min to eliminate the other extracellular vesicle types.

Transmission electron microscopy (TEM.)

To assess the morphology of the exosome, the isolated exosomes were assessed by transmission electron microscope (TEM).

Western blotting

To verify the showing of CD9 and CD81 by exosomes, the MSCs and derivative exosomes were firstly lysed in RIPA buffer (Thermo Fisher Scientific, USA). The lysate proteins were conveyed to PVDF membranes. Finally, the primary and secondary antibodies (Abcam, UK) were applied to recognize the proteins CD9 and CD81.

MTT assay

The effects of the exosomes at three

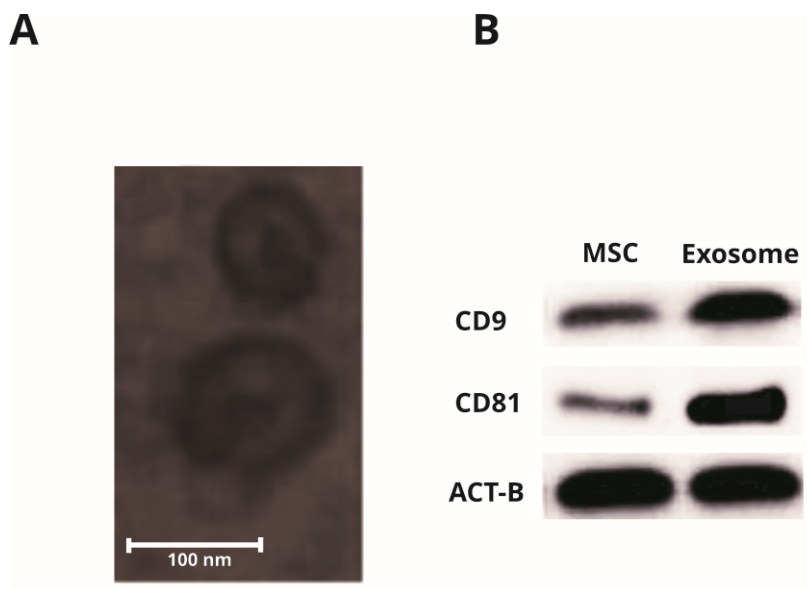

Fig. 1. The characterizing exosome using transmission electron microscopy (TEM) (A) and western blotting (CD9 and 81) (B).

Table 1. The primer pairs sequences for real-time PCR

Gene		Primer (5′-3′)
ERK	F	CTACACGCAGCTGCAGTACATC
	R	GTGCGCTGACAGTAGGTTTGA
PI3K	F	AACACAGAAGACCAATACTC
	R	TTCGCCATCTACCACTAC
AKT	F	GTGGCAAGATGTGTATGAG
	R	CTGGCTGAGTAGGAGAAC
β-actin	F	CACCCGCGAGTACAACCTTC
	R	CCCATACCCACCATCACACC

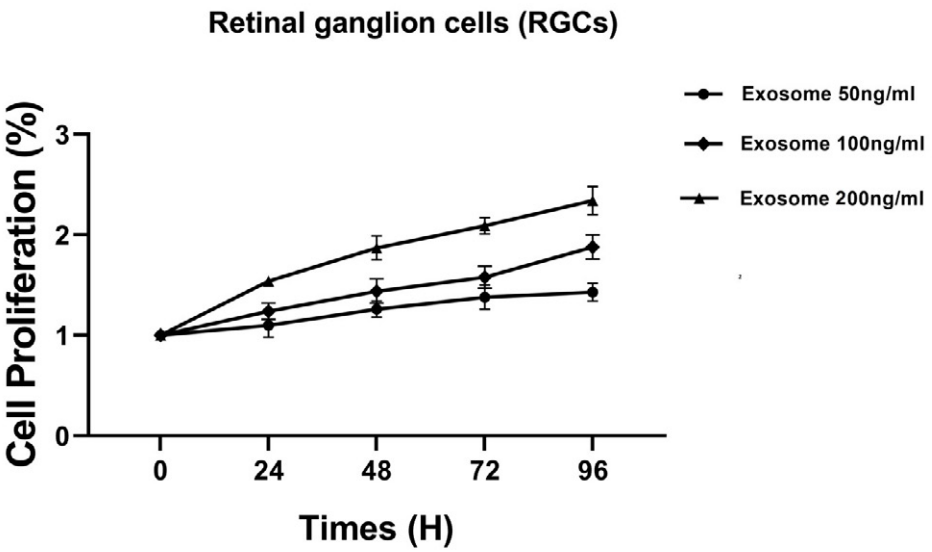

Fig. 2. The results of the MTT assay

concentrations of 50 ng/ml, 100 ng/ml, and 200 ng/ml on the RGC-5 cell line were measured using the MTT assay based on the MTT kit instructions (Abcam, UK). The 4×10^4 cells were seeded firstly in 96-well plates and incubated for 24 hours. Then we added the 50-200 ng/ml exosome, and the cells were maintained for 24-96 hours. Finally, the 10 µL of 5 mg MTT/ml medium was used, and the OD of wells was measured at 570 nm wavelengths after 4 hours of incubation.

RNA extraction and cDNA synthesis

The RNX Plus solution kit (Sinaclon, Iran) was applied to isolate the RNA from RGC-5 cells. The RGC-5 cell was lysed, and complementary DNA (cDNA) was established using the high-capacity kit (Bioneer, USA) from acquired RNA.

Real-Time PCR

To measure the expression of ERK, AKT, and PI3K at mRNA levels, Real Time-PCR was performed. In this step, the established cDNA, forward and reverse primers as listed in Table 1, distilled water, and PrimeTime™ Master Mix (Idtdna, U.S.A.) were applied.

Statistical analysis

Consequences are derived from 3 independent tests, and all are demonstrated in Mean ± SEM Student T-test was exploited in order to define the statistical alterations. Results were analyzed using Graph Pad Prism software, and the P-value < 0.05 was considered statistically significant. The β-actin gene expression was used as an internal control.

RESULTS AND DISCUSSION

MSCs-derived exosome promote RGC-5 cell proliferation

Firstly, the exosomes efficiently were characterized by TEM image and western blotting

for CD9 and CD81.

Then, the MTT assay test was managed to determine the exosome effect on RGC-5 cell proliferation. Concerning the MTT assay consequences, exosomes at concentrations of 50ng/ml, 100 ng/ml, and also 200 ng/ml enhanced the proliferation of RGC-5 cells within 24- 96 hours of treatment(Fig. 2). This effect was more evident at the concentration of 200ng/ml within 96 hours of treatment (Fig. 2). In fact, this effect was time-dependent and dose-dependent.

Other studies also have revealed that MSCs release cytokines with anti-apoptotic, neurotrophic, anti-inflammatory properties, thus exerting regenerative effect on retinal injury [19]. Also, exosomes inhibit the stimulation of inflammatory cytokines and boost the autophagy level, thereby supporting the greater survival of the photoreceptors [20, 21].

MSCs-derived exosome promoted AKT expression RGC-5 cells

The real-time PCR test was managed to evaluate AKT expression in RGC-5 cells following treatment with exosomes 50-200 ng/ml within 24- 96 hours of exposure. During 24 hours of treatment, exosomes did not affect AKT expression in RGC-5 cells at all concentrations and times($P<0.05$) (Fig. 3). However, exosomes 100 and 200 ng/ml resulted in enhancement in AKT expression at 48-96 hours of treatment ($P<0.05$) (Fig. 3). However, exosome 50 ng/ml had no effect on AKT expression at 48 and 72 hours of treatment.

Studies have shown that MSCs-exosomes could by activation of the Akt pathway [22]. Furthermore, and coworkers have signified that Akt activation resulted in enhanced viability, reduced apoptosis and autophagy of RGCs with ischemia/reperfusion injury [23].

MSCs-derived exosome enhanced PI3K expression RGC-5 cells

Real-time PCR test was managed to evaluate PI3K expression in RGC-5 cells following treatment with exosomes 50-200 ng/ml within 24- 96 hours of exposure. During 24 hours of treatment, exosomes of 50 ng/ml did not affect PI3K expression in RGC-5 cells within 24 hours of treatment ($P<0.05$) (Fig. 4). Nonetheless, exosomes 100 and 200 ng/ml promoted PI3K expression 24 hours of treatment ($P<0.05$) (Fig. 4). Besides, exosomes at three concentrations caused an enhancement of PI3K expression at 48-96 hours of treatment.

Previous studies have shown that the transduction of the PI3K/Akt pathway sustain the RGC survival after intraocular pressure (IOP) enhancement but not under normal condition [24]. Likewise, Li et al. (2008) exhibited that promoted viability of melanopsin-expressing RGCs after injury is related with the PI3 K/Akt pathway [25].

MSCs-derived exosome up-regulated ERK expression RGC-5 cells

Real-time PCR test was managed to evaluate ERK expression in RGC-5 cells following treatment with exosomes 50-200 ng/ml within 24- 96 hours of

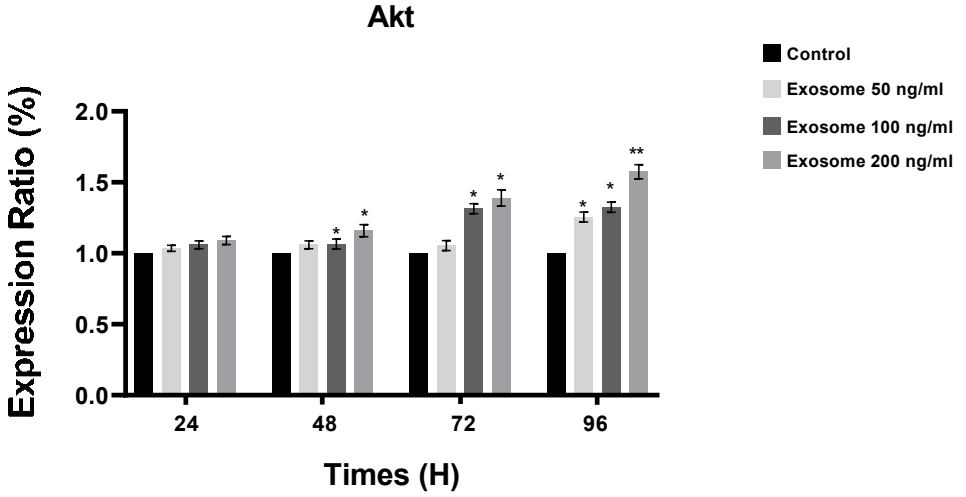

Fig. 3. The AKT expression in RGC-5 cells upon treatment with exosomes.

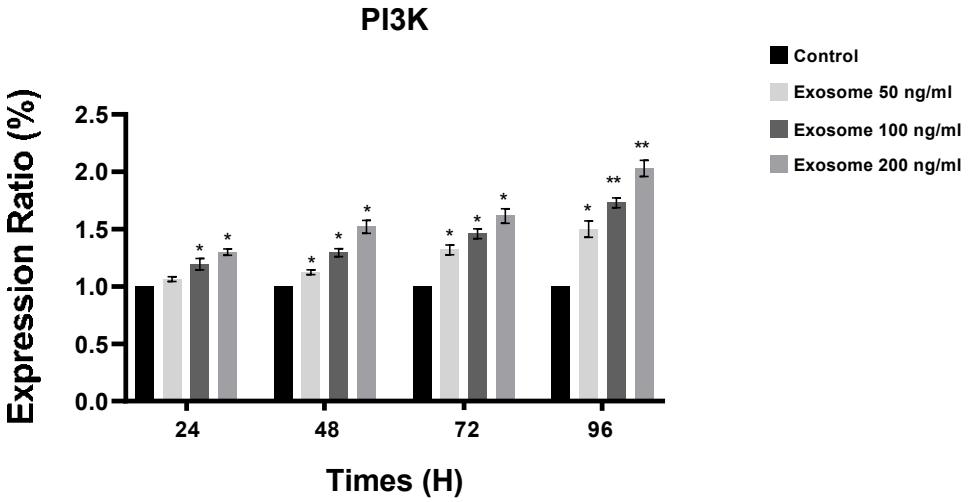

Fig. 4. The PI3K expression in RGC-5 cells upon treatment with exosomes.

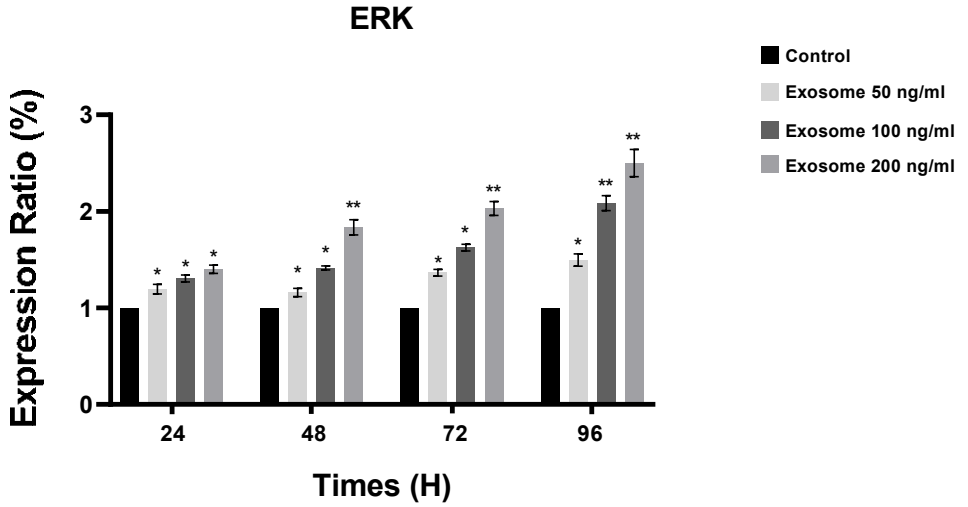

Fig. 5. The ERK expression in RGC-5 cells upon treatment with exosomes.

exposure. Accordingly, exosomes 50, 100, and 200 ng/ml treatment enhanced ERK expression within 24-96 hours of treatment ($P<0.05$) (Fig. 5).

CONCLUSION

Results revealed that MSCs-derived exosomes could improve RGC-5 cell proliferation by inducing PI3K/AKT pathways and also up-regulating ERK expression.

CONFLICT OF INTEREST

There is no conflict of interest.

REFERENCES

1. Sernagor E, Eglen SJ, Wong RO. Development of retinal ganglion cell structure and function. Prog Retin Eye Res, 2001;20(2):139-74. https://doi.org/10.1016/S1350-9462(00)00024-0
2. Sanes JR, Masland RH. The types of retinal ganglion cells: current status and implications for neuronal classification. Annual review of neuroscience, 2015;38:221-46. https://doi.org/10.1146/annurev-neuro-071714-034120
3. Boia R, Ruzafa N, Aires ID, Pereiro X, Ambrósio AF, Vecino E, et al. Neuroprotective strategies for retinal ganglion cell degeneration: current status and challenges ahead. International journal of molecular sciences, 2020;21(7):2262. https://doi.org/10.3390/ijms21072262
4. Lachowicz E, Lubiński W. The importance of the electrophysiological tests in the early diagnosis of ganglion cells and/or optic nerve dysfunction coexisting with pituitary adenoma: an overview. Documenta Ophthalmologica, 2018;137(3):193-202. https://doi.org/10.1007/s10633-018-9659-5
5. Hazirolan D, Duman M, Guler SK, Uney G, Ornek F. Retinal

ganglion cell complex and visual evoked potentials in levetiracetam treatment. Cutaneous and Ocular Toxicology, 2020;39(3):237-43. https://doi.org/10.1080/15569527.2020.1778016
6. Wójcik-Gryciuk A, Gajewska-Woźniak O, Kordecka K, Boguszewski PM, Waleszczyk W, Skup M. Neuroprotection of retinal ganglion cells with AAV2-BDNF pretreatment restoring normal TrkB receptor protein levels in glaucoma. International journal of molecular sciences, 2020;21(17):6262. https://doi.org/10.3390/ijms21176262
7. Peterson SL, Li Y, Sun CJ, Wong KA, Leung KS, de Lima S, et al. Retinal ganglion cell axon regeneration requires complement and myeloid cell activity within the optic nerve. Journal of Neuroscience, 2021;41(41):8508-31. https://doi.org/10.1523/JNEUROSCI.0555-21.2021
8. Tezel G. Multifactorial pathogenic processes of retinal ganglion cell degeneration in glaucoma towards multi-target strategies for broader treatment effects. Cells, 2021;10(6):1372. https://doi.org/10.3390/cells10061372
9. Chen W, Huang Y, Han J, Yu L, Li Y, Lu Z, et al. Immunomodulatory effects of mesenchymal stromal cells-derived exosome. Immunologic research, 2016;64(4):831-40. https://doi.org/10.1007/s12026-016-8798-6
10. Willis GR, Fernandez-Gonzalez A, Anastas J, Vitali SH, Liu X, Ericsson M, et al. Mesenchymal stromal cell exosomes ameliorate experimental bronchopulmonary dysplasia and restore lung function through macrophage immunomodulation. American journal of respiratory and critical care medicine, 2018;197(1):104-16. https://doi.org/10.1164/rccm.201705-0925OC
11. Nojehdehi S, Soudi S, Hesampour A, Rasouli S, Soleimani M, Hashemi SM. Immunomodulatory effects of mesenchymal stem cell-derived exosomes on experimental type-1 autoimmune diabetes. Journal of cellular biochemistry, 2018;119(11):9433-43. https://doi.org/10.1002/jcb.27260
12. Zhu W, Huang L, Li Y, Zhang X, Gu J, Yan Y, et al. Exosomes derived from human bone marrow mesenchymal stem cells promote tumor growth in vivo. Cancer letters, 2012;315(1):28-37. https://doi.org/10.1016/j.canlet.2011.10.002
13. Xu X, Liang Y, Li X, Ouyang K, Wang M, Cao T, et al. Exosome-mediated delivery of kartogenin for chondrogenesis of synovial fluid-derived mesenchymal stem cells and cartilage regeneration. Biomaterials, 2021;269:120539. https://doi.org/10.1016/j.biomaterials.2020.120539
14. Tan S, Wong J, Sim S, Tjio C, Wong K, Chew J, et al. Mesenchymal stem cell exosomes in bone regenerative strategies-a systematic review of preclinical studies. Materials Today Bio, 2020;7:100067. https://doi.org/10.1016/j.mtbio.2020.100067
15. Wei H, Chen J, Wang S, Fu F, Zhu X, Wu C, et al. A nanodrug consisting of doxorubicin and exosome derived from mesenchymal stem cells for osteosarcoma treatment in vitro. International journal of nanomedicine, 2019;14:8603. https://doi.org/10.2147/IJN.S218988
16. Liu W, Rong Y, Wang J, Zhou Z, Ge X, Ji C, et al. Exosome-shuttled miR-216a-5p from hypoxic preconditioned mesenchymal stem cells repair traumatic spinal cord injury by shifting microglial M1/M2 polarization. Journal of neuroinflammation, 2020;17(1):1-22. https://doi.org/10.1186/s12974-020-1726-7
17. Villamizar O, Waters SA, Scott T, Grepo N, Jaffe A, Morris KV. Mesenchymal Stem Cell exosome delivered Zinc Finger Protein activation of cystic fibrosis transmembrane conductance regulator. Journal of extracellular vesicles, 2021;10(3):e12053. https://doi.org/10.1002/jev2.12053
18. Cho BS, Kim JO, Ha DH, Yi YW. Exosomes derived from human adipose tissue-derived mesenchymal stem cells alleviate atopic dermatitis. Stem cell research & therapy, 2018;9(1):1-5. https://doi.org/10.1186/s13287-018-0939-5
19. De Ugarte DA, Alfonso Z, Zuk PA, Elbarbary A, Zhu M, Ashjian P, et al. Differential expression of stem cell mobilization-associated molecules on multi-lineage cells from adipose tissue and bone marrow. Immunology letters, 2003;89(2-3):267-70. https://doi.org/10.1016/S0165-2478(03)00108-1
20. Nakazawa T, Kayama M, Ryu M, Kunikata H, Watanabe R, Yasuda M, et al. Tumor necrosis factor-α mediates photoreceptor death in a rodent model of retinal detachment. Investigative ophthalmology & visual science, 2011;52(3):1384-91. https://doi.org/10.1167/iovs.10-6509
21. Xie J, Zhu R, Peng Y, Gao W, Du J, Zhao L, et al. Tumor necrosis factor-alpha regulates photoreceptor cell autophagy after retinal detachment. Scientific reports, 2017;7(1):1-11. https://doi.org/10.1038/s41598-017-17400-3
22. Gu H, Ji R, Zhang X, Wang M, Zhu W, Qian H, et al. Exosomes derived from human mesenchymal stem cells promote gastric cancer cell growth and migration via the activation of the Akt pathway. Molecular medicine reports, 2016;14(4):3452-8. https://doi.org/10.3892/mmr.2016.5625
23. Du H-y, Wang R, Li J-l, Luo H, Xie X-y, Yan R, et al. Ligustrazine induces viability, suppresses apoptosis and autophagy of retinal ganglion cells with ischemia/reperfusion injury through the PI3K/Akt/mTOR signaling pathway. Bioengineered, 2021;12(1):507-15. https://doi.org/10.1080/21655979.2021.1880060
24. Huang Y, Cen L-P, Luo J-M, Wang N, Zhang M-Z, Van Rooijen N, et al. Differential roles of phosphatidylinositol 3-kinase/akt pathway in retinal ganglion cell survival in rats with or without acute ocular hypertension. Neuroscience, 2008;153(1):214-25. https://doi.org/10.1016/j.neuroscience.2008.02.007
25. Li S-Y, Yau S-Y, Chen B-Y, Tay DK, Lee VWH, Pu M-L, et al. Enhanced Survival of Melanopsin-expressing Retinal Ganglion Cells After Injury is Associated with the PI3 K/Akt Pathway. Cellular and Molecular Neurobiology, 2008;28(8):1095-107. https://doi.org/10.1007/s10571-008-9286-x