Overview of Mesenchymal Stem Cell-Derived Exosomes in Ophthalmology

Subjects: Ophthalmology

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The field of mesenchymal stem cell (MSC) therapy has shown promise in treating ophthalmic diseases. However, MSC-based therapy faces limitations due to suboptimal biocompatibility, penetration, and delivery to the target ocular tissues. To overcome these challenges, researchers have turned their attention to a new aspect of MSCs - their exosomes. These extracellular vesicles possess properties similar to MSCs and can efficiently deliver therapeutic factors to ocular tissues that are typically difficult to target using conventional therapy and MSC transplantation. Exosomes, small vesicles derived from MSCs, exhibit properties such as anti-inflammatory, anti-apoptotic, and immunomodulatory that are similar to their parent cells. These characteristics make exosomes an attractive alternative to MSCs for ocular therapy. Due to their nano-size, MSC-derived exosomes have the potential to better penetrate biological barriers, such as the blood-retinal barrier, and deliver their cargo effectively to ocular tissues. In addition, their cargo is protected from degradation, leading to increased bioavailability. This makes exosomes a promising candidate for ocular drug-delivery applications.

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1. Overview of MSC-Derived Exosomes

Mesenchymal stem cell-derived exosomes (MSC-exosomes) have emerged as potential therapeutic agents in treating various ocular diseases, including traumatic, inflammatory, vascular, and degenerative conditions. Preclinical studies have demonstrated their efficacy in managing autoimmune uveitis, glaucoma, retinal injury, diabetic retinopathy, and optic neuropathy, among others [1][2][3]. Although further studies are needed, MSC-exosome therapies offer a promising new approach to treat refractory eye diseases.

2. Exosomes: Characteristics and Biogenesis

Extracellular vesicles, which play a crucial role in maintaining cellular homeostasis and are implicated in various pathologies, can be subdivided into exosomes, microvesicles, and apoptotic bodies based on size and biosynthetic pathway. Exosomes, the smallest subset of extracellular vesicles, range from 30 to 150 nm and are composed of a

cargo enclosed by a lipid bilayer. The exosomal cargo, consisting of a heterogeneous assemblage of molecules such as proteins, amino acids, metabolites, lipids, and nucleic acids (including, non-exhaustively, DNA, miRNA, mRNA, and lncRNA), is representative of the cell of origin [1][4].

Exosomes are membrane-delimited particles that play a vital role in the maintenance of cellular homeostasis and are involved in various pathologies. They are divided into exosomes, microvesicles, and apoptotic bodies based on size and biosynthetic pathway. Exosomes range in size from 30 to 150 nm, and they contain a cargo enclosed by a lipid bilayer, which is representative of the cell of origin. The exosomal cargo consists of a heterogeneous assemblage of proteins, amino acids, metabolites, lipids, and nucleic acids, including DNA, miRNA, mRNA, and lncRNA. Additionally, exosome biogenesis starts with the invagination and budding of the endosomal limiting membrane to form multivesicular bodies (MVBs), which house intraluminal vesicles. The exosomal cargo is sorted via ESCRT-dependent and ESCRT-independent pathways, with the fusion of MVBs with the plasma membrane resulting in the secretion of cup-shaped exosomes into the extracellular environment. The lipid bilayer protects the internal cargo from enzymatic degradation, preserving biological potency and integrity, allowing the exosomes to persist in the ocular structure long after release. Exosomes are optimized for long-distance transport in biological fluids, acting as mediators of intercellular communication upon internalization by target cells and the release of their protected cargo. MSCs have a far greater capacity for exosome production and secretion than cells derived from mesodermal lineages, with adipose tissue, umbilical cord, bone marrow, and corneal stroma being the primary sources of MSC-derived exosomes for the treatment of ocular diseases. Their natural occurrence in bodily fluids and parent-acquired lipid bilayer makes them highly biocompatible. Upon interaction with target cells and the liberation of the enclosed cargo into the intracellular environment, changes in gene expression and cellular function occur. MSC-exosomes are a promising therapeutic agent for a range of refractory ocular diseases, as they can serve as an effective drug delivery system.

3. Exosomes in Cellular Communication

3.1 The Transfer of Biomolecules and Modulation of Intercellular Communication

MSC-exosomes are important mediators of intercellular communication due to their unique composition of enclosed cargo, which elicits various responses in target cells. One key component is miRNAs, with over 4000 distinct types detected in exosomal cargo. The complement of miRNAs within a given MSC-exosome reflects the identity and state of the donor cell, with each source, including adipose, umbilical cord, and bone marrow-derived MSC-exosomes, housing a unique complement of miRNA types. Upon release in recipient cells, miRNAs post-transcriptionally regulate gene expression by binding to the 30UTR of mRNA. [5][6][7].

3.2. Immunomodulatory Potential in Immune-Mediated Ocular Diseases

MSC-exosomes have immense therapeutic potential due to their immunomodulatory, immunosuppresssive, pro-regenerative, pro-angiogenic, and anti-inflammatory properties. Although the eye is an immune-privileged site, immune-mediated ocular disorders can still cause significant damage to ocular tissue. MSC-exosomes have

demonstrated efficacy in treating various immune-mediated ocular disorders such as Sjögren's syndrome dry eye, corneal allograft rejection, and autoimmune uveitis by modulating the overactive immune response that characterizes these pathologies. These diseases share a common underlying mechanism, which involves the promotion of M2 macrophage differentiation and regulatory T cell development, while reducing T-lymphocyte and natural killer cell proliferation [1][8].

4. Advantages of MSC-Exosomes in Ophthalmology

MSC-exosomes offer a safer alternative to MSC transplantation, which carries risks of undesired cell differentiation and inflammation, leading to severe and irreversible complications such as vision loss, vitreous opacification, and retinal detachment. These risks can be reduced with the use of MSC-exosomes, which possess similar therapeutic benefits as MSC transplantation through the secretion of soluble paracrine factors without direct cell replacement. This approach can achieve similar efficacy with a more favorable safety profile [1][9][10].

MSC-exosomes have shown considerable promise as a novel therapeutic agent in the management of ocular diseases due to their efficacy and safety profiles. However, further study is necessary to confirm their potential, particularly in treating diseases refractory to current treatments.

MSC-exosomes can be enhanced through controlled manipulation of chemical, biological, and mechanical factors during parental cell development (MSCs) [11], and their cargo can be genetically modified. These optimization techniques are actively being researched to develop them as a novel drug delivery vehicle [1].

5. MSC-Exosome Isolation and Preservation

The most common method for isolating MSC-exosomes is differential or density-gradient ultracentrifugation [12]. However, this procedure is time-consuming, labor-intensive, and may yield impurities [13]. Other isolation methods, such as ultrafiltration, size exclusion chromatography, precipitation, and immune affinity capture, can be used instead of ultracentrifugation [13]. In addition, size-dependent and immunoaffinity-based microfluidic technologies are emerging as rapid and efficient alternatives [14]. While cryopreservation at -80°C is a typical method for long-term storage, concerns about changes in morphology and bioactivity have been raised [15]. Cryopreservation with liquid nitrogen and cryoprotective agents may provide superior preservation of exosome morphology and function [16].

6. Route of Administration of MSC-Exosomes in Ophthalmology

MSC-exosomes are typically administered through direct injection into the vitreous humor of the eye in animal models, which allows for high intraocular levels of the therapeutic agent and is well-tolerated by the recipient. However, subconjunctival and periocular injections have also been shown to be effective alternative administration

routes. Another mode of delivery is topical application as eye drops, which is minimally invasive but requires higher dosages due to the protective epithelial barrier and rapid tear turnover [17][18].

7. Bioengineering MSC-Exosomes for Enhanced Drug Delivery

MSC-exosomes have a protective lipid envelope and small size that make them a useful tool for drug delivery. Natural MSC-exosomes contain endogenous biomolecules that can be utilized for therapeutic purposes, but bioengineered exosomes have broader applications [19]. Loading of RNA, hydrophilic biological molecules, and proteins into MSC-exosomes can be achieved through transfection, electroporation, and overexpression [20]. Alternatively, drugs can be loaded directly into MSCs through transfection, and the secreted MSC-exosomes containing the drug can later be isolated [21]. Surface protein modification can improve the specificity of MSC-exosomes to their targets while limiting adverse systemic effects [20]. Targeting peptides can be modified on the exosomal surface through covalent modification, non-covalent modification, and genetic engineering to improve targeting specificity [22]. Another targeting method involves the use of iron oxide nanoparticles in conjunction with an external magnetic field to localize MSC-exosomes to a specific site of interest [22].

References

1. Yu, B.; Li, X.-R.; Zhang, X.-M. Mesenchymal Stem Cell-Derived Extracellular Vesicles as a New Therapeutic Strategy for Ocular Diseases. World J. Stem Cells 2020, 12, 178–187.
2. Cui, Y.; Liu, C.; Huang, L.; Chen, J.; Xu, N. Protective Effects of Intravitreal Administration of Mesenchymal Stem Cell-Derived Exosomes in an Experimental Model of Optic Nerve Injury. Exp. Cell Res. 2021, 407, 112792.
3. Zhang, W.; Wang, Y.; Kong, Y. Exosomes Derived from Mesenchymal Stem Cells Modulate MiR-126 to Ameliorate Hyperglycemia-Induced Retinal Inflammation Via Targeting HMGB1. Investig. Opthalmol. Vis. Sci. 2019, 60, 294.
4. Xu, H.-K.; Chen, L.-J.; Zhou, S.-N.; Li, Y.-F.; Xiang, C. Multifunctional Role of MicroRNAs in Mesenchymal Stem Cell-Derived Exosomes in Treatment of Diseases. World J. Stem Cells 2020, 12, 1276–1294.
5. Chen, T.S.; Lai, R.C.; Lee, M.M.; Choo, A.B.H.; Lee, C.N.; Lim, S.K. Mesenchymal Stem Cell Secretes Microparticles Enriched in Pre-MicroRNAs. Nucleic Acids Res. 2010, 38, 215–224.
6. Lai, R.C.; Tan, S.S.; Teh, B.J.; Sze, S.K.; Arslan, F.; de Kleijn, D.P.; Choo, A.; Lim, S.K. Proteolytic Potential of the MSC Exosome Proteome: Implications for an Exosome-Mediated Delivery of Therapeutic Proteasome. Int. J. Proteomics 2012, 2012, 971907.

7. Glover, K.; Mishra, D.; Singh, T.R.R. Epidemiology of Ocular Manifestations in Autoimmune Disease. Front. Immunol. 2021, 12, 744396.
8. Seo, Y.; Kim, H.-S.; Hong, I.-S. Stem Cell-Derived Extracellular Vesicles as Immunomodulatory Therapeutics. Available online: https://www.hindawi.com/journals/sci/2019/5126156/ (accessed on 14 February 2023).
9. Kuriyan, A.E.; Albini, T.A.; Townsend, J.H.; Rodriguez, M.; Pandya, H.K.; Leonard, R.E.; Parrott, M.B.; Rosenfeld, P.J.; Flynn, H.W.; Goldberg, J.L. Vision Loss after Intravitreal Injection of Autologous “Stem Cells” for AMD. N. Engl. J. Med. 2017, 376, 1047–1053.
10. Sun, H.; Pratt, R.E.; Hodgkinson, C.P.; Dzau, V.J. Sequential Paracrine Mechanisms Are Necessary for the Therapeutic Benefits of Stem Cell Therapy. Am. J. Physiol. Cell Physiol. 2020, 319, C1141–C1150.
11. McKelvey, K.J.; Powell, K.L.; Ashton, A.W.; Morris, J.M.; McCracken, S.A. Exosomes: Mechanisms of Uptake. J. Circ. Biomark. 2015, 4, 7.
12. Tang, Y.; Zhou, Y.; Li, H.-J. Advances in Mesenchymal Stem Cell Exosomes: A Review. Stem Cell Res. Ther. 2021, 12, 71.
13. Elliott, R.O.; He, M. Unlocking the Power of Exosomes for Crossing Biological Barriers in Drug Delivery. Pharmaceutics 2021, 13, 122.
14. Jia, Y.; Ni, Z.; Sun, H.; Wang, C. Microfluidic Approaches Toward the Isolation and Detection of Exosome Nanovesicles. IEEE Access 2019, 7, 45080–45098.
15. Zhang, Y.; Bi, J.; Huang, J.; Tang, Y.; Du, S.; Li, P. Exosome: A Review of Its Classification, Isolation Techniques, Storage, Diagnostic and Targeted Therapy Applications. Int. J. Nanomed. 2020, 15, 6917–6934.
16. Zhang, Z.; Mugisha, A.; Fransisca, S.; Liu, Q.; Xie, P.; Hu, Z. Emerging Role of Exosomes in Retinal Diseases. Front. Cell Dev. Biol. 2021, 9, 643680.
17. Moisseiev, E.; Anderson, J.D.; Oltjen, S.; Goswami, M.; Zawadzki, R.J.; Nolta, J.A.; Park, S.S. Protective Effect of Intravitreal Administration of Exosomes Derived from Mesenchymal Stem Cells on Retinal Ischemia. Curr. Eye Res. 2017, 42, 1358–1367.
18. Zhou, T.; He, C.; Lai, P.; Yang, Z.; Liu, Y.; Xu, H.; Lin, X.; Ni, B.; Ju, R.; Yi, W.; et al. MiR-204–Containing Exosomes Ameliorate GVHD-Associated Dry Eye Disease. Sci. Adv. 2022, 8, eabj9617.
19. Wang, J.; Chen, D.; Ho, E.A. Challenges in the Development and Establishment of Exosome-Based Drug Delivery Systems. J. Control. Release 2021, 329, 894–906.
20. Sun, Y.; Liu, G.; Zhang, K.; Cao, Q.; Liu, T.; Li, J. Mesenchymal Stem Cells-Derived Exosomes for Drug Delivery. Stem Cell Res. Ther. 2021, 12, 561.

21. Bian, B.; Zhao, C.; He, X.; Gong, Y.; Ren, C.; Ge, L.; Zeng, Y.; Li, Q.; Chen, M.; Weng, C.; et al. Exosomes Derived from Neural Progenitor Cells Preserve Photoreceptors during Retinal Degeneration by Inactivating Microglia. J. Extracell. Vesicles 2020, 9, 1748931.
22. Yu, M.; Liu, W.; Li, J.; Lu, J.; Lu, H.; Jia, W.; Liu, F. Exosomes Derived from Atorvastatin-Pretreated MSC Accelerate Diabetic Wound Repair by Enhancing Angiogenesis via AKT/ENOS Pathway. Stem Cell Res. Ther. 2020, 11, 350.