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Adipose Stem Cell Secretome for Corneal Endothelial Regeneration: Ki-67 Expression, Hexagonality, and CV Restoration after Ultrasound Exposure in Phacoemulsification

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ABSTRACT

Corneal endothelial cell (CEC) damage, particularly following cataract surgery, remains a significant challenge in ophthalmology, as CECs have limited regenerative capacity. Adipose-derived stem cell (ASC) secretome, a rich mixture of cytokines, growth factors, exosomes, and non-coding RNAs, has shown promise in enhancing tissue repair and regeneration. Ki-67, a well-established marker of cell proliferation, serves as an indicator of the regenerative potential of CECs. Hexagonality, a measure of the uniform, regular cell shape crucial for maintaining the structural and functional integrity of the corneal endothelium. The coefficient of variation (CV) of endothelial cell size is a sensitive marker of endothelial dysfunction. The underlying mechanisms by which ASC secretome mediates these regenerative effects include the inhibition of oxidative stress, modulation of TGF-β signaling, and regulation of mitochondrial function. The secretome's influence on mitochondrial integrity is particularly significant, as mitochondrial dysfunction is a key factor contributing to endothelial senescence and loss of function. Additionally, the paracrine signaling mediated by ASC exosomes plays a crucial role in enhancing cellular communication and tissue repair. This review discusses the clinical implications of ASC secretome in corneal endothelial regeneration and its potential as a non-invasive, donor-independent therapeutic option. By highlighting recent advancements and mechanisms, the review provides a comprehensive understanding of how ASC secretome could be utilized to address the challenges of corneal endothelial dysfunction, offering new prospects for treating endothelial cell loss and improving surgical outcomes in cataract patients.

Keywords: *Corneal endothelial cells; adipose stem cell secretome; Ki-67; hexagonality; coefficient of variation; phacoemulsification*

INTRODUCTION

The cornea is a transparent, avascular tissue that serves as the primary refractive surface of the eye. Its critical role in vision is underscored by the importance of its structural and functional integrity, which is maintained by the corneal endothelial cells (CECs). This function is vital for maintaining corneal transparency and preventing edema. However, the regenerative capacity of CECs is limited, particularly when endothelial cell loss occurs due to trauma or surgical procedures, such as cataract surgery (1,2).

The loss of endothelial cells is a common complication following phacoemulsification, a procedure widely used for cataract removal. Studies have shown that between 5% and 20% of endothelial cells can be lost within the first 1-3 months post-surgery (3,4). Traditional treatment options for endothelial cell loss, such as corneal transplantation, are not always ideal due to challenges related to donor availability, the risk of immune rejection, and the complications associated with surgery (5). Therefore, there is a growing need for alternative, non-invasive therapies aimed at regenerating corneal endothelial cells. One promising approach is the use of adipose-derived stem cells (ASCs), which are readily available and have demonstrated regenerative potential across a variety of tissues.

ASCs are multipotent cells that secrete a variety of bioactive molecules, including cytokines, growth factors, and exosomes, collectively referred to as the ASC secretome. This secretome has been shown to promote tissue regeneration by enhancing cell proliferation, migration, and survival, as well as modulating inflammation and immune responses (6). This review aims to provide a comprehensive analysis of the impact of ASC secretome on corneal endothelial regeneration. Specifically, it will focus on the effects of ASC secretome on Ki-67 expression, hexagonal morphology, and CV, all of which are critical indicators of CEC health and function. In addition, this review will explore the underlying molecular mechanisms

through which ASC secretome mediates these effects, offering insights into the therapeutic potential of ASC secretome for treating corneal endothelial dysfunction and improving the outcomes of cataract surgery.

Corneal endothelial wound healing

Corneal endothelial wound healing involves a complex series of cellular events that aim to restore the integrity and function of the corneal endothelium after injury. Unlike other epithelial tissues, corneal endothelial cells (CECs) do not undergo mitosis in vivo; instead, they repair the wound through cell migration and enlargement. This process is critical for maintaining corneal transparency and function. Endothelial wound healing is characterized by the migration and enlargement of neighboring healthy CECs to cover the damaged area. This process is driven by various signaling pathways, including those mediated by growth factors such as Basic Fibroblast Growth Factor (bFGF), Hepatocyte Growth Factor (HGF), and Transforming Growth Factor-beta (TGF-β) (Joyce & Zhu, 2004; Joko et al., 2013). The migration of CECs is facilitated by changes in the cytoskeleton, especially the actin filaments, which allow for cellular protrusions (filopodia and lamellipodia) that help the cells move across the damaged area (7).

One key feature of endothelial healing is the phenomenon known as endothelial-to-mesenchymal transition (EnMT). During this process, CECs lose their typical hexagonal morphology and acquire fibroblast-like characteristics. This transition plays a crucial role in the closure of the wound, but it can also result in fibrosis if not properly regulated. The expression of markers such as fibronectin and vimentin increases during EnMT, while junctional proteins like E-cadherin are downregulated (8).

Several molecular pathways are involved in endothelial wound healing. Key pathways include: 1. **PI3K/Akt Pathway**: This pathway plays a crucial role in regulating cell survival, proliferation, and migration during the wound healing process (9). 2. **TGF-β Signaling**: TGF-β is a multifunctional cytokine that regulates both the healing and fibrotic responses. While TGF-β helps to close the wound by promoting cell migration, prolonged activation of this pathway can lead to fibrosis and loss of endothelial function (10). 3. **Wnt/β-Catenin Signaling**: This pathway is involved in regulating the balance between cell proliferation and differentiation. β-Catenin activation has been associated with both the promotion of CEC proliferation and the induction of EnMT (11).

The figure below summarizes the key signaling pathways involved in corneal endothelial wound healing, including the PI3K/Akt, TGF-β, and Wnt/β-Catenin pathways, and their role in cell migration, proliferation, and fibrosis.

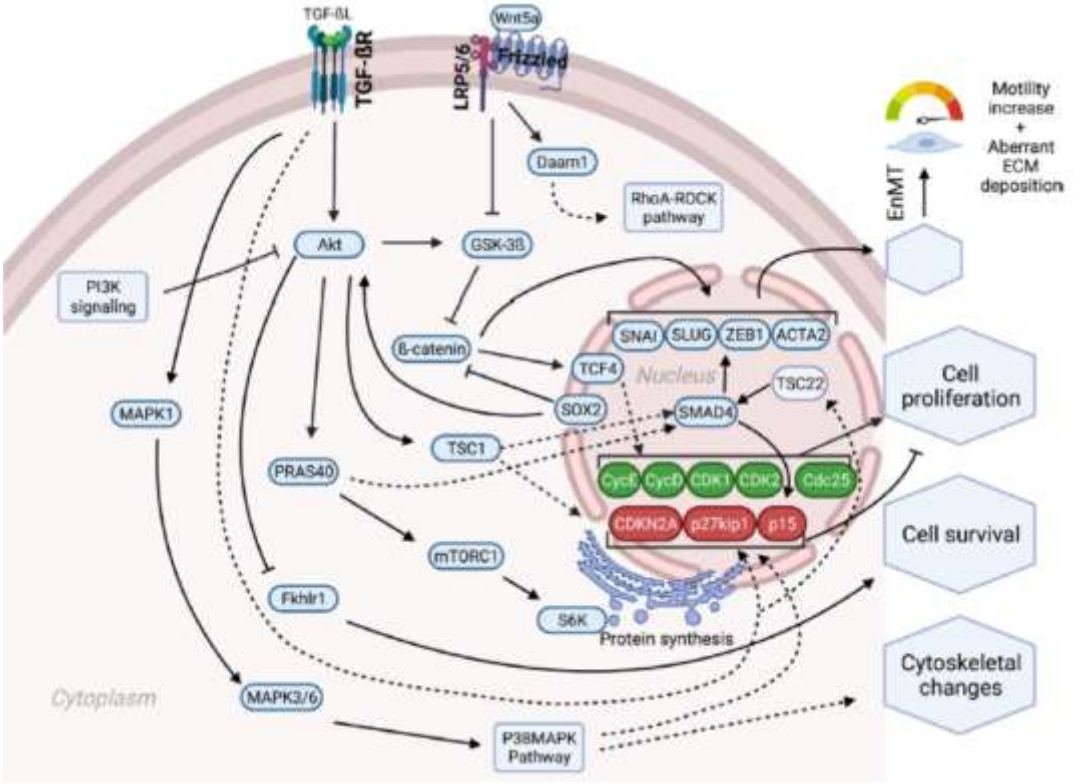

Figure 1. The signaling pathways involved in corneal endothelial wound healing, including the PI3K/Akt, TGF-β, and

Wnt/β-Catenin pathways, and their role in cell migration, proliferation, and fibrosis (9).

Adipose Stem Cell Secretome

Adipose-derived mesenchymal stem cells (ASCs) are multipotent stem cells capable of differentiating into various cell types, including osteocytes, adipocytes, and chondrocytes (12). These cells are primarily isolated from adipose tissue, a highly abundant and easily accessible source of stem cells, making ASCs an attractive candidate for regenerative therapies (13).

The ASC secretome consists of extracellular vesicles, including exosomes and ectosomes, growth factors, cytokines, non-coding RNAs (such as microRNAs), and lipids. These components work synergistically to promote cellular proliferation, migration, and survival, and to modulate immune responses (14). Notably, exosomes, which are small extracellular vesicles released by ASCs, contain molecular cargo—such as microRNAs—that can regulate gene expression in recipient cells, facilitating tissue regeneration (6). Exosomes derived from ASC secretome have demonstrated significant potential in promoting tissue repair by enhancing cell proliferation and reducing fibrosis (14).

The regenerative effects of ASC secretome are primarily attributed to its ability to promote cell proliferation, reduce oxidative stress, and enhance cell migration. ASC secretome also plays a crucial role in preventing fibrosis and endothelial-to-mesenchymal transition (EnMT), a process that can hinder corneal endothelial regeneration (6). In particular, ASC secretome has shown promise in restoring corneal endothelial cell (CEC) function by modulating oxidative stress, improving mitochondrial function, and reducing the risk of fibrosis in damaged (14).

Ki-67 Expression and Proliferation of Endothelial Cells

Ki-67 is a nuclear protein that is expressed in proliferating cells during the G1, S, G2, and M phases of the cell cycle. Its expression is absent in quiescent cells (G0 phase), making it a reliable marker for evaluating cellular proliferation (15). The expression of Ki-67 is widely used to assess the regenerative capacity of corneal endothelial cells (CECs) following injury.

ASC secretome has been shown to significantly promote cell proliferation in various tissues, including the corneal endothelium. Studies indicate that ASC secretome increases Ki-67 expression in CECs, suggesting that it can stimulate endothelial cell division and regeneration (16). The presence of growth factors such as VEGF, FGF, and HGF in the secretome may enhance the progression of the cell cycle, thereby boosting endothelial cell proliferation (17).

Hexagonality in Corneal Endothelial Cells

Hexagonal morphology is a defining feature of healthy corneal endothelial cells. In a normal cornea, the majority of CECs are arranged in a regular hexagonal pattern, which is essential for maintaining the structural integrity and function of the corneal endothelium. Disruption of this hexagonal arrangement, often due to aging, trauma, or surgical injury, results in impaired endothelial function, contributing to conditions like corneal edema (18).

ASC secretome has been shown to restore the hexagonal pattern of CECs following injury. This restoration is attributed to the growth factors and cytokines present in the ASC secretome, which help re-establish the normal cell-cell junctions and cytoskeletal dynamics that are critical for maintaining the hexagonal arrangement of CECs (19). Restoration of hexagonal morphology is vital for the corneal endothelium to maintain its barrier and pump function, which is necessary to prevent corneal swelling and loss of transparency (20).

Coefficient of Variation (CV) in Corneal Endothelial Cells

The coefficient of variation (CV) is a statistical measure of the variation in cell size within a population. In the context of corneal endothelial cells, a lower CV indicates a more uniform and functional population of cells, whereas a higher CV is indicative of disorganized cell growth, often seen in aging or damaged corneas. Increased CV is associated with poor endothelial function and a higher risk of corneal (19,21).

ASC secretome has been demonstrated to reduce CV in corneal endothelial cells, thereby improving cell uniformity and function. This effect is achieved by promoting cell proliferation and restoring the normal morphology of CECs, leading to a more uniform cell population that is essential for maintaining corneal transparency and preventing edema (6).

Mechanisms of ASC Secretome in Corneal Endothelial Regeneration

The regenerative potential of adipose-derived stem cell (ASC) secretome in corneal endothelial regeneration involves a multifaceted interplay of signaling pathways, cellular responses, and microenvironmental modulation. Several principal mechanisms underpin these effects:

1. Activation of PI3K/Akt Pathway

The ASC secretome is rich in growth factors such as VEGF, FGF, HGF, and IGF-1, which activate the PI3K/Akt signaling pathway. Activation of this pathway enhances cell survival, proliferation, and migration. Akt activation promotes G1 to S phase transition in the cell cycle by regulating downstream effectors, enabling corneal endothelial cells, which are

otherwise arrested in the G1 phase, to re-enter the cell cycle. This mechanism is crucial because human corneal endothelial cells have a limited capacity for proliferation in vivo but retain latent regenerative potential if appropriately stimulated (9,22).

2. Modulation of TGF-β Signaling and Prevention of EnMT

While TGF-β is essential for tissue repair, its overactivation induces endothelial-to-mesenchymal transition (EnMT), promoting fibrosis and loss of endothelial function. The ASC secretome delicately modulates TGF-β signaling. It inhibits pathological TGF-β-induced responses by: Reducing SMAD2/3 activation, Increasingproduction of regulatory molecules such as decorin and TSG-6, which suppress fibrogenic responses and Preventing EnMT by maintaining expression of tight junction proteins like ZO-1 and Na^+/K^+-ATPase, critical for endothelial barrier function (11,14).

3. Role of MicroRNAs (miRNAs) in Secretome

The ASC secretome is particularly enriched in non-coding RNAs, especially miRNAs such as miR-21 (Suppresses apoptosis by downregulating PDCD4 and inhibits fibrosis by targeting SMAD7), miR-24 (Reduces oxidative stress-induced senescence by modulating mitochondrial integrity and preventing apoptosis), and miR-26 (Targets negative regulators of the PI3K/Akt pathway like PTEN, promoting survival and proliferation). These miRNAs collectively act to promote endothelial cell proliferation, inhibit apoptosis, modulate inflammation, and suppress fibrotic transformation, thereby enhancing regenerative outcomes (6,14).

4. Reduction of Oxidative Stress and Mitochondrial Protection

The ASC secretome also combats oxidative stress, a major contributor to corneal endothelial cell senescence and death post-injury. It enhances antioxidant defenses, protects mitochondrial function by transferring mitochondrial DNA, lipids, and proteins through extracellular vesicles (exosomes) and reduces mitochondrial depolarization and inhibits p53-dependent apoptotic pathways. Maintaining mitochondrial homeostasis is vital for sustaining the energy-intensive pump function of corneal endothelial cells (14,16).

5. Inhibition of Autophagy-Mediated Cell Damage

Excessive autophagy can exacerbate EnMT and cell death during stress. ASC secretome inhibits stress-induced autophagic pathways, likely through modulation of TGF-β and p53 signaling, thereby preserving endothelial monolayer integrity (14).

6. Immunomodulatory Effects

The secretome contains anti-inflammatory cytokines such as IL-10 and TSG-6, which downregulate inflammatory responses that otherwise delay wound healing. By reducing inflammation, the secretome creates a permissive environment for regeneration (13).

Discussion

Adipose-derived stem cell (ASC) secretome has emerged as a promising therapeutic approach for corneal endothelial regeneration, offering a potential solution to the limitations of traditional treatments such as corneal transplantation. ASC secretome is rich in bioactive molecules, including growth factors, cytokines, and exosomes, that can promote tissue repair by enhancing cell proliferation, migration, and survival, while also reducing inflammation and oxidative stress (6).

In the context of corneal endothelial cells (CECs), ASC secretome has shown significant effects on key functional parameters, including Ki-67 expression, hexagonal morphology, and the coefficient of variation (CV) of cell size. Ki-67, a well-established marker of proliferation, was shown to increase in CECs treated with ASC secretome, suggesting its potential to stimulate cell division and regeneration (16). Similarly, ASC secretome has been demonstrated to restore the hexagonal arrangement of CECs, a characteristic feature of healthy endothelial cells (18). Furthermore, ASC secretome was found to reduce the CV of endothelial cells, which is an indicator of improved cell uniformity and function, essential for maintaining corneal transparency (20).

The potential of ASC secretome for corneal endothelial regeneration offers several clinical implications. ASC secretome has the advantage of being a cell-free therapy, which eliminates the challenges associated with donor tissue availability and immune rejection seen in traditional corneal transplantation (17). Furthermore, ASC secretome can be produced in large quantities, stored, and transported more easily than living cells, making it a highly accessible and scalable option for clinical use.

The therapeutic effects of ASC secretome in corneal endothelial regeneration are particularly relevant for patients undergoing cataract surgery, where endothelial cell loss is a common complication. By promoting endothelial cell proliferation and restoring the normal morphology and function of CECs, ASC secretome could significantly improve the outcomes of cataract surgery, reducing the risk of corneal edema and the need for corneal transplantation (3).

Challenges and Limitations

Despite its promising therapeutic potential, several challenges remain in the use of ASC secretome for corneal endothelial regeneration. One major limitation is the variability in the quality and composition of ASC secretome, which may differ depending on the source and method of preparation (13). Additionally, while ASC secretome has been shown to promote cell proliferation and migration in vitro, further studies are needed to evaluate its long-term effects and safety in vivo, particularly in clinical settings.

Another challenge is the need to better understand the mechanisms through which ASC secretome exerts its effects on CECs. While much has been learned about the role of ASC secretome in promoting cell proliferation and migration, the specific signaling pathways involved—such as the modulation of TGF-β, PI3K/Akt, and Wnt/β-catenin signaling—require further investigation to optimize therapeutic strategies and improve clinical outcomes (9,11).

Future Directions

Future research should focus on elucidating the precise mechanisms by which ASC secretome influences CEC function, particularly its effects on mitochondrial health, oxidative stress modulation, and the regulation of fibrosis. Additionally, clinical trials are needed to assess the safety and efficacy of ASC secretome in patients with endothelial dysfunction, including those who have undergone cataract surgery.

Advancements in the delivery methods of ASC secretome, such as intravitreal injections or topical applications, should also be explored. These methods could offer non-invasive options for delivering ASC secretome to the corneal endothelium, potentially improving patient compliance and therapeutic outcomes (16).

Conclusion

In conclusion, ASC secretome represents a promising and innovative approach to corneal endothelial regeneration. Its ability to promote cell proliferation, restore hexagonal morphology, and reduce CV in endothelial cells makes it an ideal candidate for treating endothelial dysfunction caused by trauma or surgery. While further research is needed to fully understand the underlying mechanisms and optimize clinical applications, ASC secretome has the potential to become a valuable therapeutic tool in ophthalmology, particularly in the management of endothelial cell loss following cataract surgery.

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