Overcoming Challenges in the Clinical Translation of MSC-Exosomes

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Contributor: Varun Srikanth , Kevin Yang Wu , Laura Gorwill , Shrieda Jain , Kyungwook Kim , Marjorie Carbonneau , Simon D. Tran

MSC-based therapy for ophthalmic diseases has limitations in biocompatibility, penetration, and delivery. To address these challenges, researchers are exploring MSC exosomes, which possess similar properties to MSCs and efficiently deliver therapeutic factors to difficult-to-target ocular tissues. Research has shown that MSC-derived exosomes may offer significant advantages over traditional MSC-based therapies in regenerative medicine. By using exosomes, practitioners can avoid potential risks associated with MSC-centered therapies such as allogeneic immunological rejection, unwanted differentiation, and obstruction of small vessels caused by intravenous MSC injection. These benefits are critical for optimizing treatment outcomes.

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1. Overcoming Challenges in the Clinical Translation of MSC-Exosomes

Despite the potential of MSC-exosome therapies, unresolved challenges such as non-uniformity in isolation and purification, unclear mechanisms of action, low-yield capacity, and unstandardized large-scale production protocols still need to be addressed [1]. However, the most significant obstacle to clinical transformation is the lack of standardized quality assessment criteria and the heterogeneity of exosomal products. The International Society for Cell and Gene Therapy has developed criteria differentiating MSCs sourced from various targets [2], but more comprehensive criteria are needed to address parental MSC quality, MSC-exosome quality, and potential for ex vivo expansion. Whereas, heterogeneity hinders product quality and management, reducing reproducibility in both in vivo and in vitro contexts.

2. Assessment of Parental MSCs as Proxy Indicator of MSC-Exosome Quality

To ensure high-quality MSC-exosome products, a standardized donor selection and screening approach is needed. Studies have investigated various assessment metrics, such as in vitro characteristics, donor demographics, EV

sourcing, and genetic biomarkers, to differentiate high- and low-quality MSCs. These criteria can be used as proxy indicators of MSC-exosome quality and inform clinical decision-making. High-potency MSCs are believed to be more effective, but a comprehensive assessment panel is required for informed decision-making.

Samsonraj et al. (2015) investigated factors affecting bone marrow-derived mesenchymal stem cells (BMSCs) and their in vivo tissue regeneration potential [3]. MSCs with high colony-forming unit-fibroblasts (CFU-F) showed an increase in small-sized cells with lengthened telomeres, and high growth capacity performed better on ectopic bone-formation assays. STRO-1+ and nestin+ MSCs expressing PDGFR-α were predictive of greater high-growth and colony formation capacity. Additionally, a global gene expression analysis revealed that low-growth BMSCs had more maturation-based cellular processes, while high-growth BMSCs had more proliferation-based processes.

Sathiyanathan et al. (2020) conducted a transcriptomic analysis of genetic biomarkers in BMSCs to understand their effect on scalability [4]. The study identified glutathione S-transferase theta 1 (GSTT1) as the most differentially expressed gene, with low-growth BMSCs showing a fifty-fold greater expression of the gene. High-growth BMSCs were found to have a genomic deletion of GSTT1, leading to its repression. The subsequent double-blind study revealed that GSTT1-null BMSCs demonstrated greater growth and self-renewal capacity, longer telomeres, and higher total cell count and CFU-F efficiency, making GSTT1 a meaningful genetic biomarker for BMSC scalability. Therefore, GSTT1 status in donors could be used as a rapid assessment tool for harvesting-related decisions and to inform usage in clinical applications.

Boulestreau et al. (2020) reviewed the impact of aging on MSC quality [5]. Age-related changes to MSCs result in the loss or increased dysfunctionality of stem cell functionality. While the precise etiology of the relationship between MSC functionality and age remains unclear, some notable findings have been consistently reported in the literature. First, the proliferative and clonogenic capacity of BMSCs is negatively associated with age [6], with specific cell-surface markers such as decreased CD146 expression and upregulated CD296 linked to late-passage MSCs. Second, reactive oxygen species and consequent oxidative stress are higher in aging MSCs. Some studies have investigated possible treatments for reversing age-related changes to MSCs. For example, melatonin is protective against oxidative stress and senescence [7], and selective inhibitor ML141 decreased CDC42 protein activity in aging MSCs [8]. Siegal et al. (2013) found that younger female donors exhibited higher clonogenicity and increased proliferative rates and had more favorable BMSCs than other donor demographics [9].

Ulum et al. (2018) reported that high BMI associated with obesity predicts reduced MSC quality [10]. In donors with higher BMI, BMSCs exhibited functional impairment, including a significant reduction in osteogenic differentiation and slower proliferation rates. These changes were accompanied by a higher proportion of senescent cells and a decline in critical stromal adhesion proteins and MSC markers. The proposed mechanism for this reduction in quality is the promotion of ER stress-related genes (ATF4 and CHOP) due to protein misfolding in obese individuals. This leads to the unfolded protein response (UPR) and subsequently, stem cell dysfunction. To address these challenges, potential solutions have been investigated, including treatment with TUDCA and 4-PBA, both of which regulate UPR- and ER stress-related proteins, improve osteogenic and adipogenic differentiation, and

prevent UPR dysfunction. These treatments may attenuate the obesity-related decrease in MSC quality, as the global obesity rate continues to rise.

Li et al. (2021) compared the benefits and drawbacks of allogeneic and autologous MSCs as extracellular vesicle sources [11]. Although the literature is still debating the best source, allogeneic MSCs are increasingly used in MSC-based therapies due to their favorable safety profile, high accessibility, and donor selection. However, potential immune rejection and donor heterogeneity may limit its application. Autologous MSC sourcing is safer as it is sourced directly from the patient, but the primary disadvantages are long-time availability and potential disease-candidate genes. Additionally, the local microenvironment can influence the functional properties of MSCs; thus, tissue-derived MSCs from a potentially harmful microenvironment may not be ideal. To optimize treatment outcomes and patient satisfaction, clinicians should thoroughly communicate the safety and efficacy profile of each approach to their patients.

3. Overcoming the Barriers of MSC-Exosome Heterogeneity

Parental and exosomal heterogeneity hinder the quality and management of MSC-exosome products, reducing their reproducibility in in vivo and in vitro contexts [12], leading to heterogeneous results. Different parental sources have shown varying therapeutic effects. For example, BMSC-derived MSC-exosomes are four times superior to ADSC-derived exosomes in terms of angiogenicity, while endometrial-derived MSC-exosomes are significantly better than both. ADSC-derived exosomes have been shown to produce more cardio-protective factors such as VEGF and HGF. Regarding immunomodulation, BMSC- and ADSC-derived exosomes can induce M2 polarization of macrophages, but BMSCs show a 3.2-fold increase in CD206 expression compared to only 1.5-fold in ADSCs. Given such heterogeneity, further research is needed to explore the nuanced effects of different parental sources and corresponding exosomes to determine the optimal sourcing and extraction protocol for patients. Additionally, comprehensive criteria for assessing the quality of parental MSCs, MSC-exosomes, and their potential for ex vivo expansion are needed for exosome-based therapies to be clinically successful [1][2].

Kou et al. (2020) suggest a solution to the problem of source and exosome product heterogeneity: extracting exosome products from human pluripotent stem cells (hPSCs)-derived MSCs [12]. Clinical trials exploring the use of hPSC-derived MSC-exosomes in patients with refractory graft-versus-host disease (GVHD) have shown promising results, with significant improvements in cutaneous chronic GVHD and rejection following abdominal organ transplantation. The hPSC-MSCs have a higher passage number and can produce higher exosomal yields than traditional MSCs. Additionally, hPSC-MSCs have improved secretion and amplification abilities, resulting in higher quality MSC-exosome products and cost-efficient, large-scale production potential.

Varderidou-Minasian and Lorenowicz (2020) highlight that the paracrine signaling of MSC therapy is crucial, rather than engraftment or differentiation capabilities of the transplanted MSCs [13]. Therefore, the qualitative and quantitative characteristics of MSC-secretomes, including EVs and exosomes, should be considered during quality assessment. Recent advancements in cell culture technology indicate that 3D culture systems better replicate in vivo conditions than traditional 2D cultures, preserving critical traits such as morphology, functionality, and

structure, which are important for proliferative and differentiation capacity, ultimately enhancing exosomal efficacy [14][15].

Ni Su et al. (2017) found that extracellular matrices with oriented fibers, compared to non-oriented, were more conducive to the release of anti-inflammatory and angiogenic-promoting factors [16]. Therefore, 3D culturing is a better option for replicating in vivo conditions than traditional static adherent 2D cultures, which hamper exosomal efficacy [14]. 3D culturing can be divided into material-free and material-supported, with the latter more conducive for cell-to-cell connectivity and signaling. Examples of material-free cell cultures include hydrogel-assisted 3D cultures and scaffold-free suspension cultures, while material-supported cultures include hollow fiber bioreactors. The latter have demonstrated a 19.4-greater yield than 2D cultures within shorter culture periods. MSC-exosomes harvested from 3D cell cultures have also been shown to improve several conditions in rat models, including improved angiogenicity and the proliferation and migration of endothelial cells in the context of injury repair.

Preconditioning of MSCs is another approach to improve the quality of MSC-exosome products [14]. Hypoxic preconditioning, involving low-oxygen tension, enhances the MSCs' proliferative capacity and genetic stability, leading to improved migratory and paracrine capacity. The extracted exosomes from these preconditioned MSCs, called Hyp-MSC-Exos, have shown therapeutic benefits in various diseases, such as CNS issues and diabetic wound healing. Another preconditioning protocol, cytokine preconditioning, involves the stimulation of cytokines and inflammatory factors, which increases the paracrine efficiency of MSC-exosomes. Exposure to TNF-α, IL-1β, or IFN-γ promotes the release of exosomes with increased anti-inflammatory properties and the presence of inflammation-suppressing miRNAs. Additionally, chemical and physical preconditioning approaches, such as treatment with metformin or monochromatic blue light (451 nm), can also produce similar effects.

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