

Is the regulation of SIRT1 by miRNA-34a the key to mesenchymal stem cell survival?

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Mesenchymal stem cells (MSCs) are currently being used clinically to treat cardiovascular diseases, including ischemic heart disease, heart failure, and peripheral vascular disease (1,2). To date, these trials have proven MSCs to be a safe and effective option for the improvement of vascular function, reduction of scar size, and reversal of remodeling in heart failure (3,4). Despite the positive data being collected, there are still a number of challenges that reduce the effectiveness of MSC cell therapy. Cell survival and engraftment in the hostile microenvironment of the diseased myocardium severely limits MSC regenerative potential. Research focusing on MSC biology, including the identification of genes and molecules that regulate aspects of regeneration, is critical towards the establishment of effective cell production and delivery strategies (5,6).

Gene therapy and the manipulation of protein expression via genetic editing and targeted microRNA (miRNA) technologies has become a promising avenue for the enhancement of cellular regenerative potential (7). The delivery of genetically modified MSCs to ischemic hearts in pre-clinical studies has advanced our understanding of which genes could potentially improve the efficacy of MSC therapy. Overexpression of genes such as extracellular superoxide dismutase (ecSOD) and vascular endothelial growth factor (VEGF) in MSCs has improved survival and tissue-repair when cells are injected into sites of ischemic injury (8,9). Similarly, modification of miRNAs via lentiviral induced expression or silencing has made it possible to target entire pathways rather than individual genes. Delivery of MSCs transduced to overexpress miRNA-126 enhanced ischemic angiogenesis through the activation of AKT and

ERK regulated signaling pathways (10). Together, these studies have provoked further investigation to establish the optimal gene targeting systems that will optimize MSC therapeutic applications.

In an intriguing study published in *Stem Cell Research and Therapy*, Zhang *et al.* demonstrated a novel role for miRNA-34a in the regulation of apoptosis and senescence by MSCs via the silent information regulator 1 (SIRT1)-mediated pathways (11). As a well-studied miRNA, the down-regulation of miRNA-34a promotes proliferation, increases stress resistance, and promotes cell migration in a variety of cell types. These outcomes are particularly appealing to a cell-therapy application where cell survival and tissue integration is at risk. The therapeutic advantages of modifying miRNA-34a expression are further showcased through the feasibility of specific alteration using oligonucleotide mimetic or inhibition. Particularly in cancer therapy, induced expression of miRNA-34a safely reduced tumor growth and survival (12). In this context, the authors describe that MSC apoptosis and decline of cell proliferation correlates with miRNA-34a upregulation. This response serves as a promising therapeutic target in MSCs because recent studies have demonstrated a consistent role of miRNA-34a in cell cycle progression, particularly due to its regulation of genes such as p53, c-kit, SIRT1, and Notch (13). Based on these findings, the authors formulated and tested the hypothesis that overexpression of miRNA-34a exacerbates hypoxia and serum starvation-induced apoptosis and senescence in MSCs.

The combination of hypoxia and serum starvation is a common inducer of MSC apoptosis, and is a reliable model to test molecular mechanisms that may be altered

upon cell injection into hypoxic tissue (14). Zhang *et al.* used this model to test the potency of miRNA-34a overexpression and silencing on cell apoptosis via the detection of annexin-V positive cells. As expected, those MSCs engineered to overexpress miRNA-34a exhibited a greater amount of apoptosis upon hypoxic serum starvation conditions, while silencing miRNA-34a significantly reduced this effect. These findings were then expanded upon in order to identify the key molecular players involved in the miRNA-34a response.

SIRT1 is an established and key target of miRNA-34a due to its endogenous regulation of anti-apoptotic and pro-proliferative pathways. Acting as a NAD-dependent protein deacetylase, SIRT1 has been described as a longevity factor, targeting transcription factors such as forkhead box proteins (FOX) and p53, while tightly regulating cellular resistance to oxidative damage (15). However, it had not been previously shown whether miRNA-34a manipulation and subsequent regulation of SIRT1 affects MSC biology. The authors used SIRT1 gene silencing paired with miRNA-34a inhibition to test the involvement of SIRT1 in the miRNA-34a-mediated apoptotic response. The authors demonstrated that miRNA-34a regulates SIRT1 and the downstream pro-apoptotic factor FOXO3a. Moreover, silencing SIRT1 expression abolished the anti-apoptotic effects of inhibiting miRNA-34a. This result was confirmed by the alteration of apoptotic markers caspase-3, poly ADP ribose polymerase (PARP), and mitochondrial cytochrome C. Lastly, markers of DNA damage and senescence decreased with miRNA-34a inhibition, providing further evidence for the multifaceted effects of miRNA-34a in MSCs.

Although these findings serve as a promising first step toward establishing a genetic strategy designed to effectively preserve the viability of MSCs delivered into the ischemic myocardium, many questions are still left to be resolved. The broad range of RNA targets of miRNA-34a may be beneficial or problematic, and the long-term effect of miRNA-34a inhibition on MSCs has not been explored. The goal of MSC therapy is for transplanted MSCs to provide lasting reparative effects on myocardial structure and function by engrafting into the myocardium. miRNA-34a overexpression vectors are currently being investigated in translational models of cancer to halt unregulated cell proliferation and cancer metastasis (16). Whether the permanent inhibition of miRNA-34a in MSCs is a safe option that does not pose tumorigenic risk needs to be determined. Additionally, genes that influence MSC

differentiation and angiogenesis, two important factors for the regenerative response, are known targets of miRNA-34a. Expression of platelet-derived growth factor receptor (PDGFR), which is down regulated by miRNA-34a, plays a significant role in MSC mediated vasculogenesis (17,18). It would be important to investigate how the regulation of multiple pathways by miRNA-34a would impact the *in vivo* differentiation and tissue integration of MSCs. Lastly, the experiments performed by Zhang *et al.* were all done in 5% oxygen (11), a level that is hypoxic compared to standard laboratory culture conditions (21% oxygen) but not compared to the physiological oxygen levels in the mammalian heart. It has been reported that ischemia in the heart could decrease oxygen levels from a physiologic 5% to a low of 1–3% oxygen (19). To confirm the effectiveness of miRNA-34a silencing in ischemia, similar experiments would need to be conducted at lower oxygen tensions.

In summary, Zhang *et al.* have presented a novel and promising genetic targeting strategy that could enhance the effectiveness of MSC therapy in ischemic heart disease. Their findings demonstrate that miRNA-34a inhibition results in greater viability of MSCs in an *in vitro* model of hypoxia and serum starvation, warranting further investigation in an *in vivo* translational model.

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Footnote

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Conflicts of Interest: Dr. Hare has a patent for cardiac cell-based therapy; he holds equity in Vestion Inc., maintains a professional relationship with Vestion as a consultant and member of the Board of Directors and Scientific Advisory Board, and is a shareholder in Longeveron LLC. The other authors have no conflicts of interest to declare.

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