

Role of Microparticles as Messengers Enhancing Stem Cell Activity After Genetic Engineering

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Despite significant therapeutic advances over several decades, myocardial infarction remains the most common cause of death worldwide. In this context, cell-based therapy was considered a promising novel strategy for regeneration of healthy, functionally integrated, myocardial tissue. Unfortunately, the clinical trials using cell-based therapy did not always confirm the preclinical experimental results.^{1,2} Therefore, in the last decade, the efforts were focused on studying the complex molecular and cellular mechanisms contributing to improved myocardial remodeling and function after stem cell transplantation.³

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Because cell-based therapy has been proposed, various cell types were tested in transplantation experiments.^{4–10} Among all, autologous bone marrow-derived CD34⁺ stem cells demonstrate a major benefit, showing favorable impact on angiogenic growth, without the toxicity and inflammatory response observed in nonautologous cell-based therapy.^{11,12} However, insufficient number of donor stem cells, low cell viability, and inefficient expansion techniques limit the effects of this therapy. In this regard, overexpressing angiogenic growth factors in transplanted cells has been reported to be beneficial by attenuating myocardial ischemia and preserving the heart function.^{13–17}

In this issue of *Circulation Research*, Mackie et al investigate the mechanisms of preserving the heart function after transplantation of CD34⁺ cells overexpressing sonic hedgehog (Shh) protein, a well-established angiogenic factor.¹⁸ After inducing myocardial infarction in nude mice, the authors transplanted the Shh-modified CD34 (CD34^{Shh}) cells, which significantly preserved cardiac function, reduced infarct size, and increased angiogenesis within the infarct border zone, as compared to the empty vector-transfected or -untransfected CD34⁺ cells. The short-term retention of CD34⁺ cells, as well as short-term secretion of Shh after

myocardial infarction, are required for the positive benefit. As a potential mechanism, Mackie et al found that CD34^{Shh} cells are able to deposit greater amount of Shh in exosomes and transfer them to other cell types, activating the Shh signaling pathway in recipient cells. Among all cell types, only CD34⁺ cells preferentially store Shh in exosomes. Moreover, the Shh exosomal transfer is able to induce Shh specific signaling not only in endothelial cells, but also in fibroblasts. Exosomal transfer of functional Shh protein from Shh-expressing to recipient cells represents a novel and original mechanism of cell-based therapy. To fully understand the complex mechanisms mediating the recovery of the heart after an ischemic insult, one needs to consider not only effects on cells directly involved, but also collateral effects on the inflammatory reaction and the paracrine secretion of remote cells. These aspects were not fully elucidated in this study and should be further investigated. Likewise, it is not clear whether these exosomes or microparticles also transport other factors potentially modulating these processes.

Because low viability and inefficient expansion are important limitations of stem-cell therapies,¹ enhancing stem-cell survival in the hostile environment is the main goal of current cell-based therapy.¹⁹ Genetic reprogramming of the transplanted stem cells allows them to serve as a source of growth factors, initiating intracrine, autocrine, and paracrine effects and augmenting the activity of endogenous cells. This dual approach, using stem-cell transplantation and genetic engineering to potentiate stem cell activity for myocardial repair, seems to be a reliable method and is currently successfully used in many experimental settings.^{17–21} As an example, overexpressing stromal derived factor-1 (CXCL12), an important chemokine in cardiac development, protects the myocardium and sustains the repairing after myocardial infarction,²² by promoting regeneration and angiogenesis through recruitment of progenitor cells.^{23,24} Notably, the vasoregenerative and angiogenic potential of angiogenic early outgrowth cells can be enhanced by the transfer of the CXCL12 receptor CXCR4 through platelet microparticles.²⁵

Similarly, the Shh signaling pathway, also essential in embryonic development, is upregulated in mammalian fibroblasts and cardiomyocytes after myocardial infarction, reducing fibrosis and cardiac apoptosis, and preserving the left ventricular function.^{26–28} Unfortunately, because of the complexity and interdependency of postmyocardial infarction events, the current knowledge does not provide sufficient information about the relative contributions of a direct rescue of existing myocardium versus the angiogenic activity or recruitment of endogenous repair mechanisms. In the present study, however, Makie et al suggest that Shh positively modulates all these processes, demonstrating a high therapeutic potential. In

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addition to the cardioprotective effects, shared with other cytokines (tumor necrosis factor- α , hypoxia-inducible factor) and chemokines (CCL2, CXCL12, macrophage inhibitory factor),³ Shh is a well-established angiogenic morphogen, enhancing neoangiogenesis.²⁹ Moreover, it can potentiate the CXCR4 antagonist AMD3100-induced progenitor-cell mobilization after myocardial infarction, improving cardiac recovery.³⁰ However, Shh overexpression in different cells leads to different outcomes through different mechanisms.³¹ It seems that only CD34⁺ cells were able to deposit Shh in exosomes, and only after genetic modification but not after treatment with the Shh protein.¹⁸ Furthermore, CD34⁺ cell-derived exosomes carry Shh protein and transfer it to other cells, activating Shh-signaling pathway in recipient cells. The exosomes are cell-derived microvesicles or microparticles composed of a phospholipid bilayer enclosing cytosolic components,³² playing a pivotal role in cell-to-cell communication.²⁰ TNF- α and angiotensin-2, which are upregulated after myocardial infarction,³³ stimulate endothelial microparticle generation.³⁴ Circulating microparticles from patients with myocardial infarction correlate with endothelial dysfunction and can predict the evolution of the cardiac diseases.³⁵ In addition to proteins, the microparticles can also transport and transfer mRNAs, eg, associated with the PI3K/AKT signaling pathway, to activate an angiogenic program in endothelial cells³⁶ or microRNAs, namely miR-126, which can promote endothelial regeneration in a CXCL12-dependent mechanism.³⁷ The transfer of gene products from cell-to-cell explains stem cell function without the need of transdifferentiation into tissue cells.²⁰

From a clinical perspective, the findings presented by Mackie et al point toward the advantages of applying the concept of microparticles as therapeutic devices for the treatment of myocardial injury: the ability to carry and deliver specific content to the target, the possibility for an in vitro expansion of the microparticles, and the avoidance of damaging clearance mechanisms after transplantation. Thus, the potential of microparticles in enhancing stem cell activity after genetic engineering may provide a key tool for developing novel therapeutic strategies to improve cardiac remodeling, function of the heart, and prognosis of patients after myocardial infarction. However, only gaining further insights into the complexity of the molecular interactions may allow the identification of responsible mechanisms, their connections, and how these mechanisms can be modulated for development of reliable therapies.

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Disclosures

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