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The importance of understanding the molecular mechanism of stem cell-induced cardiac tissue repair

“...a patient is predisposed to stem cell-based healing as either a ‘healer’ or a ‘scarrer’. If they are a healer, they recover from end-organ injury but enhance dysplastic tissue/tumor growth, whereas if they are a scarrer, they scar in response to tissue injury and are prone to atherosclerosis and ischemic events but do not support tumor growth.”

KEYWORDS: acute myocardial infarction ■ chemokines ■ chronic heart failure ■ endogenous stem cell repair ■ stem cells

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The potential for stem cell repair of injured tissue has garnered great hope for the prevention and treatment of cardiac dysfunction. Early studies suggested that the direct transplantation of bone marrow-derived stem cells resulted in improved cardiac function in acute myocardial infarction (AMI) and chronic heart failure through the direct regeneration of lost cardiac myocytes [1,2]. In the early 2000s, there was significant controversy regarding the validity of cardiac differentiation of stem cells following transplantation and engraftment in acutely injured myocardium [3]. At that time, lost in the controversy regarding cardiac myocyte regeneration was the fact that stem cell transplantation in AMI and chronic heart failure led to improvements in cardiac function and remodeling [1,4–7].

Over the ensuing several years there was increasing understanding of the importance of the paracrine effects of stem cell-based tissue repair. Work from Dzau and colleagues nicely demonstrated that conditioned media from mesenchymal stem cells (MSCs) could induce the same benefits as engraftment of the MSCs themselves and that the effects of conditioned media could be modulated and enhanced by manipulation of the MSC secretome [8]. Mayoraga and colleagues further refined the signaling pathways responsible for cardiac protein expression in MSCs, ultimately demonstrating that cardiac protein expression in MSCs in response to TGF β was not associated with cardiac regeneration but actually enhancement of the MSC secretome [9,10].

We participated in developing the multipotent adult progenitor cell (MAPC) [11] to prevent cardiac dysfunction in AMI. In our

preclinical studies we demonstrated significant preservation of cardiac function and improvement in neovascularization in the post-AMI heart despite the limited survival of the MAPC beyond 6 weeks after AMI [12,13]. These data further supported that the benefits of adult stem cell therapy were not linked to cardiac regeneration but rather paracrine factor release and enhancement of endogenous tissue repair. The fact that nonsurviving stem cells could induce a clinical benefit was demonstrated in the first-in-man MAPC study in which MAPCs were delivered to the adventitia of patients 2–5 days after primary stenting for first AMI [14]. In this study we demonstrated a significant dose-dependent increase in ejection fraction at 4 months after AMI that was sustained for 1 year despite the lack of MAPC survival.

Against the backdrop of the field's focus on the need for stem cells as a therapeutic agent, in 2000 we proposed the following hypothesis: stem cell-based repair of injured tissue is a natural process that, owing to dysregulation of the reparative system rather than lack of stem cells, does not lead to significant clinical benefit. At that time we sought to define the molecular regulators of endogenous stem cell repair of injured tissue. The impact of individual factors on benefits observed through the delivery of stem cell therapy has begun to be defined through the following two studies. We initially identified SDF-1 as a key regulator of stem cell recruitment to the heart following AMI [15] and demonstrated that the overexpression of SDF-1 first through cell-based gene transfer and later standalone gene transfer in ischemic cardiomyopathy led to improvements in cardiac dimensions and function [15–18]. In subsequent studies

we identified a critical role for MCP-3 as a MSC homing factor [19], as well as roles for disabled-2 and NOTCH signaling in endogenous stem cell repair [9,10]. Recently, using mice that lack cardiac myocyte and cardiac stem cell CXCR4 expression, we demonstrated that the release of SDF-1 by MSCs is critical for MSC-induced inhibition of cardiac myocyte death and enhancement of cardiac stem cell recruitment [20]. These data demonstrate that an important mechanism of the effect of adult stem cell therapy is activation of endogenous stem cell repair and support our prior hypothesis that one benefit of adult stem cell therapy is the temporal realignment of SDF-1 secretion with CXCR4 receptor expression by end-organ cells (cardiac myocytes) and endogenous tissue-specific stem cells (cardiac stem cells) [21]. The importance of temporal alignment of the SDF-1–CXCR4 axis has also been shown in the eye in response to retinal detachment [22,23].

“...one benefit of adult stem cell therapy is the temporal realignment of SDF-1 secretion with CXCR4 receptor expression by end-organ cells...”

In 2011 we began to test the hypothesis that defining the molecular mechanisms of stem cell-based tissue repair could lead to novel therapeutics in a Phase I clinical trial that sought to re-establish SDF-1 expression in the myocardium of patients with New York Heart Association (NYHA) Functional Classification class III chronic heart failure due to a history of myocardial infarction (NCT01082094). In this study we delivered 5, 15 or 30 mg of JVS-100, a DNA plasmid encoding human *SDF-1* [18]. In this invasive first-in-man study, a placebo control was not an option but the 5 mg dose was anticipated to be nontherapeutic. In this recently published study we observed [24]:

- Overexpression of SDF-1 for approximately 17 days [17] in the heart is safe;
- Resolution of peri-infarct ischemia in the 15 and 30 mg cohorts at 4 months;
- Clinically meaningful improvements in 6 min walk, quality of life and NYHA classification at 4 months, sustained to 12 months especially in the 30 mg cohort;
- Stabilization of cardiac function in the 15 and 30 mg cohorts;
- A decline in the clinical and functional status at 12 months in the 5 mg cohort.

As a small (17 patient) Phase I study these data should be considered as proof of concept at best. Two double-blind, randomized, placebo-controlled studies measuring the effects of SDF-1 overexpression in patients with chronic heart failure (NCT01643590) and critical limb ischemia (NCT01410331) are ongoing and will further validate our hypothesis.

It is well recognized that one of the advantages of stem cell therapies is that multiple indications can be treated with the same preparation. Based on this observation, arguably one feature of defining a relevant mechanism of stem cell-based repair is that the mechanism is active in multiple disease or injury states. Consistent with this, we have recently demonstrated that inducing SDF-1 overexpression improves surgical wound healing leading to faster wound closure with significantly less scarring [25]. These observations are now being tested clinically in a study of patients undergoing median sternotomy for open-heart surgery (NCT01657045).

Stem cell-based therapies have tremendous potential to prevent and treat the morbidity and mortality associated with chronic disease and end-organ dysfunction. Clinical trials testing the safety and effects of stem cells to prevent and treat cardiac dysfunction have overwhelmingly demonstrated the safety of the approach. Some trials have demonstrated meaningful and potentially clinically important findings [26,27], whereas others have failed to demonstrate benefits [28–30]. It is clear that adult stem cells trials will move forward testing the multiple different approaches that have developed the cardiovascular regenerative medicine space [31]. The overwhelming data suggest that these adult stem cells lead to activation of an endogenous stem cell-based repair system; thus, the question is will future approaches utilize cells at all? In medicine when we want to induce the immune system we do not transfuse B and T cells, we deliver adjuvants. The data to date suggest that there are adjuvants that prime endogenous stem cell repair. Whether the target is SDF-1 or other chemokines/growth factors remains to be determined. Importantly, the field, funding agencies and peer-review system need to move from hand-waving away the issues of mechanism of action and move to rigorous hypothesis-based science that defines the biology of endogenous stem cell repair.

Perhaps just as important as developing therapies that can lead to improved clinical outcomes is the potential to greatly expand our understanding of pathophysiology more broadly. A

decade ago when we proposed stem cell-based repair was a natural but clinically inefficient process, a corollary to our hypothesis was that mammals have evolved to scar instead of heal in an attempt to minimize tumor. While still an unproven hypothesis, we would be remiss if we did not note that upregulation of SDF-1, MCP-3 and NOTCH, and downregulation of disabled-2 – all factors that in our hands enhance stem cell function – are all associated with the enhancement of tumor survival. These observations have led us to propose the concept that a patient is predisposed to stem cell-based healing as either a ‘healer’ or a ‘scarrer’. If they are a healer, they recover from end-organ injury but enhance dysplastic tissue/tumor growth, whereas if they are a scarrer, they scar in response to tissue injury and are prone to atherosclerosis and ischemic events but do not support tumor growth. While an unproven hypothesis at this time, it is testable. Through defining the molecular mechanisms associated with stem cell-based tissue repair these types of

interesting and transformative hypotheses can be generated.

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