Although advances in pharmacotherapy and interventional cardiology have greatly reduced the mortality and morbidity following myocardial infarction (MI), there remains an ongoing need for innovative cell transplantation techniques that can reverse cardiac ventricular remodeling post-MI. Despite the barriers facing the application of cell-based interventions (1,2), a growing number of preclinical studies and clinical trials have demonstrated the safety of a variety of adult stem cell types. Mesenchymal stem cells (MSCs) are one of the most well-studied cell types used in regenerative medicine (3). Human MSCs have the capability for self-renewal and can differentiate into various mesenchymal and non-mesenchymal tissues and are currently under evaluation in clinics for the treatment of cardiovascular diseases, such as MI (2). However, different isolation and expansion techniques result in remarkable differences in their proliferation capacity and differentiation potentials (4). Furthermore, clinical applications of MSCs require a large number of expanded cells; however, many studies have reported that expanded MSCs are heterogeneous and contain a significant portion of senescent cells (4). Thus, the development of novel culture methods for expanding homogenous and non-senescent MSCs without the loss of proliferation, stemness, and multi-differentiation abilities would be of great value for this research field.

Culturing MSCs under hypoxic conditions before transplantation, also known as hypoxic preconditioning, is one strategy for improving the homogeneity and properties of expanded cells. Preclinical animal studies have demonstrated the therapeutic advantages of MSCs cultured under hypoxic conditions (referred to as hypoxic MSCs) over those cultured under ambient conditions (referred to as normoxic MSCs) in diseases, such as bony defects (5), tendon tear (6), stroke (7), and hindlimb ischemia (8). Hypoxic MSCs have also been applied in animal models to treat diseases, such as osteoarthritis (9), fulminant hepatitis (10), and early atherosclerotic lesion (11). Although hypoxic MSCs have previously been evaluated in various disease models with positive outcomes, it remains to be determined how this technique will function in future clinical applications to treat MI. A prior study on the application of hypoxic MSCs to reduce MI complications reported improvements in left ventricular function in pigs that received allogeneic hypoxic MSCs compared with pigs that received allogeneic normoxic MSCs (12). Although this large animal study demonstrated the safety and efficacy of hypoxic MSCs for treating chronic ischemic heart failure, there is a lack of primate studies demonstrating the superiority of hypoxic MSCs over normoxic MSCs. The most recent study by Hu et al. was the first study in non-human primates and the first large animal study of acute MI (13). The authors tested whether hypoxic MSCs were more effective than normoxic MSCs in the treatment of myocardial injury in a randomized trial in which cynomolgus monkeys (Macaca fascicularis) were subjected to acute MI. MSCs were isolated from the bone marrow, labeled with green-fluorescent protein...
by lentiviral transduction, and cultured under hypoxic conditions (0.5% $O_2$) or under ambient conditions (21% $O_2$) for 24 h. Aliquots of cells (1×10^7 cells per heart) were then intramyocardially injected into the infarcted hearts of allogeneic recipients at five different sites in the border zone 30 min after the induction of left anterior MI. Infarct size and left ventricular function 90 days after MI were measured using magnetic resonance imaging and positron emission tomography, and monkeys treated with hypoxic MSCs, but not normoxic MSCs, had significant improvements in infarct size and function compared with monkeys treated with the control vehicle. Hypoxic MSC transplantation was also associated with increases in endogenous cardiomyocyte survival and proliferation, glucose uptake, vascular density, and engraftment of the transplanted cells but did not cause long-term arrhythmogenic complications, which were observed in non-human primate hearts treated with human embryonic-stem-cell-derived cardiomyocytes (14). Because Hu et al. did not observe neocardiomyogenesis and the engraftment rates for normoxic and hypoxic MSCs were low, the benefit of hypoxic MSC transplantation was likely due to the upregulation of transcription factors or the secretion of paracrine factors that stimulated endogenous cytoprotective or regenerative mechanisms. These factors include hypoxia-inducible factor-1, angiopoietin-1, and erythropoietin.

Hypoxic culture has been considered to be a solution for the problems facing MSC-based regenerative medicine (15). The reasons for the use of hypoxic culture with MSCs are as follows: in long-term culture, hypoxia can inhibit senescence, increase the proliferation rate, and enhance differentiation potential along the different mesenchymal lineages (16). More importantly, hypoxic culture increases the expression of pluripotency transcription factors in MSCs, which in turn upregulate Dnmt1, thereby inhibiting the expression of p16 and p21, and the developmental markers or lineage genes (17). Hypoxia also modulates the paracrine effects of MSCs, causing the upregulation of various secretable factors, including vascular endothelial growth factor and IL-6 (18). Hypoxia also plays an important role in the mobilization and homing of MSCs, primarily by inducing SDF-1-CXCR4 (19). Furthermore, hypoxic MSCs have immune privilege and are less likely than normoxic MSCs to be rejected by the immune system of allogeneic recipients (8).

Aside from the technology used to expand cells, successful cell transplantation depends on many factors, including CMC data preparation, IND, and an excellent design for preclinical and clinical trials. For example, the timing of cell transplantation is the key to success. The Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial using intracoronary bone marrow mononuclear cell delivery to treat MI found that delivery 5 to 7 days after acute MI resulted in a greater improvement in global cardiac function compared to an earlier delivery time (20). The mode of cell delivery is important in determining initial engraftment and retention. In a direct head-to-head comparison among different cell delivery methods, myocardial engraftment or retention was highest when peripheral blood mononuclear cells were delivered intramyocardially (11%±3%) compared to intracoronary (2.6%±0.1%) or intravenous delivery (3.2%±1%) (11). Hu et al. used intramyocardial injection as a delivery route, which may have also contributed to the success of their preclinical trial.

In conclusion, Hu et al. demonstrated that hypoxia-preconditioned allogeneic MSCs pass the test for use in myocardial repair in non-human primates. MSCs may indeed be an ideal cell source for cardiac repair. Before MSC therapy can be used to treat MI, it will be necessary to identify the critical components that determine successful cellular therapy, as well as the biological mechanisms underlying their effects.

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### Footnote

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