There is no cure for ischemic heart disease; therefore, limiting damage and salvaging viable myocardium are the current gold standard for patient care. In recent years, much interest has surrounded the idea of cell-based myocardial regeneration as a therapeutic alternative to conventional treatment regimens. However, success in human trials using a variety of stem cells or progenitor-like cells has been limited. This has left the field to ponder how this approach can be improved. It seems as though the ideal candidate cell type should effectively maintain the stem cell/progenitor population and efficiently differentiate into functional cardiomyocytes. Admittedly, this is no easy task. The reprogramming of somatic cells to induced pluripotent stem cells, or even directly to cardiomyocyte-like cells, is remarkable and is expected to have lasting effects on stem cell biology and medicine in general. Yet this reprogramming approach to organ regeneration remains limited in its current state at least partly because of the 1:1 ratio between starting material and finished product.

Asymmetrical cell division is generally defined as the generation of distinctly destined daughter cells from a single mother cell and is a hallmark of stem and progenitor cells. There are two described mechanisms by which this phenomenon occurs. Intrinsically mediated asymmetrical cell division occurs when the mother cell segregates cell fate determinants nonrandomly. This ensures that one daughter cell receives a disproportionate amount of “stem cell” factors compared to the other daughter cell, which is more likely to differentiate and lose its stemness. Niche-mediated asymmetrical cell division utilizes the surrounding extracellular environment, which is critical to maintaining the stem cell population, to “favor” one daughter cell over the other by placing it in close contact with the niche, thereby prolonging its stemness.

Asymmetry can also occur at the DNA level through the nonrandom segregation of sister chromosomes. The mechanism by which this occurs, however, is still controversial. One theory, known as the “immortal strand” hypothesis, was put forth by Cairns in 1975 and proposes that template DNA strands cosegregate (and are retained in stem cell populations) as a way to limit replication-associated DNA damage, thereby preserving DNA integrity for long durations and many cell divisions. Findings in support of this hypothesis have been reported in various cell types by several independent groups, yet there is still no empirical evidence linking this observation to mutation rates or cancer. An alternative theory is the “silent sister” hypothesis, which proposes that asymmetrical cell division and cell fate are codirected by epigenetic differences between sister chromatids. It argues that distinct marks at centrosomal DNA and perhaps other regions direct nonrandom segregation of chromatids during mitosis. It also predicts differences in postmitotic gene expression as a mechanism to influence cell fate. However, for both theories, the mechanism by which cells distinguish between sister chromatids remains to be established.

Pim-1 is a highly conserved serine/threonine kinase and an established downstream target of the cardioprotective kinase Akt that promotes survival in a variety of cell types, including cardiomyocytes. Pim-1 is constitutively active in its nascent state and is regulated through transcription, translation, and degradation. Pim-1 also regulates cell-cycle progression and chromatin dynamics, thereby modulating proliferation. In the heart, Pim-1 expression is increased in response to injury and protects against myocardial infarction. Myocardial Pim-1 transgenic mice have increased levels of Bcl-2, Bcl-xL, and p-Bad, healthier mitochondria, and are protected against cardiac injury.
Pim-1 expression also regulates CPCs. Increasing Pim-1 levels via lentiviral transduction were shown to promote proliferation, differentiation, and survival of CPCs. After myocardial infarction, Pim-1–transduced CPCs caused reduced infarct, stimulated increased differentiation into cardiomyocytes, and increased neovascularization. The ability of Pim-1 to promote increased CPC self-renewal and differentiation, while avoiding unwanted off-target effects resulting from gene manipulation, makes it an extremely attractive target for optimizing cell-based therapies for heart disease.

The current study by Sundararaman et al provides mechanistic insight into how Pim-1 can accomplish these beneficial effects in CPCs by focusing on asymmetrical chromosome segregation (ACS). First, the authors are able to quantify the rate of ACS in CPCs cultured from adult mouse hearts. Second, they demonstrate that CPCs transduced with Pim-1 are nearly twice (4.95% versus 9.19%) as likely to undergo ACS compared to enhanced green fluorescent protein-transduced CPC controls. As a corollary, if increased Pim-1 expression promotes ACS, then does depletion of Pim-1 reduce it, ie, is endogenous Pim-1 required for ACS? This pertinent question remains unanswered.

The observation that <5% of the CPC population practices ACS raises the question of heterogeneity among CPCs and stem cells in general. If differences exist between subpopulations of CPCs, then how then can we identify and isolate those subpopulations better-suited for regeneration? Perhaps novel markers, in addition to those already used, will help to identify progenitors with an increased propensity for proliferation, self-renewal, and differentiation. Recent work has demonstrated that CPCs positive for the insulin-like growth factor 1 (IGF-1) receptor have increased regenerative capacity, suggesting that subpopulations of CPCs can be isolated and may prove to be more effective for cell-based therapy.

Two complimentary methods are used by Sundararaman et al to quantify ACS in CPCs: the label release assay and the label retention assay. For the label release assay, cells are mitotically synchronized and incubated (pulsed) with BrdUrd for a short period (6 hours, one expected mitotic event) so that the BrdUrd label is uniformly distributed/incorporated. Cells are then chased in medium lacking BrdUrd, arrested during cytokinesis, and BrdUrd intensity is measured in the binucleated cells. If ACS has occurred, then one daughter cell should contain the majority (>70%) of BrdUrd signal compared to the sister. An absolute ratio (100:0) is not used, because previous work has demonstrated that not all chromosomes segregate nonrandomly.

The label retention assay also requires synchronization of cells before incubation with BrdUrd label; however, cells are grown in BrdUrd medium for an extended period (18–25 days) to ensure that all DNA is labeled in all cells. CPCs are then chased in BrdUrd-free medium for two rounds of mitosis. If ACS occurs, then the stem/progenitor cell population will retain the BrdUrd label (70:30). Conversely, symmetrical chromosome segregation will give rise to an evenly diluted BrdUrd signal intensity. Although this method allows for the effective identification and quantification (via signal intensity) of asymmetrical versus symmetrical chromosome segregation, it is not without limitations. Importantly, the fixation and labeling requirements interfere with the ability to study the functional significance of ACS in living cells.

IGF-1 has been used to improve the efficacy of cell-based therapies for treatment of myocardial ischemia. Delivery of CPCs in the presence of IGF-1 was shown to increase myocardial regeneration and improve outcomes. Mechanically, IGF-1 has been shown to increase survival, self-renewal, and proliferation of pluripotent cell types. It can also promote the differentiation of progenitor cells to cardiomyocytes, smooth muscle cells, and endothelial cells. The multifaceted benefits of IGF-1 are rather unique and make it an attractive candidate for improving cell-based therapy. IGF-1 is a potent activator of Akt and has been shown to increase Akt localization in the nucleus. Because nuclear Akt can activate Pim-1 to elicit cardioprotection, it is possible that IGF-1–mediated activation of Akt causes increased Pim-1 signaling in CPCs and promotes ACS.

The article by Sundararaman et al provides exciting new insight into the dynamics of chromosome segregation and the ability of Pim-1 to enhance ACS in CPCs. It also gives rise to many still unanswered questions. It will be extremely interesting to next determine the functional significance of this asymmetrical enhancement. Does this translate to a larger pool of stem cell–like progenitors? Will this, in turn, lead to more differentiated cardiomyocytes? Does ACS serve to limit mutagenesis of DNA in stem and progenitor cells, thereby prolonging their stemness and delaying aging? Similarly, do CPCs undergoing ACS have an increased regenerative potential versus symmetrical CPCs and, if so, could this difference be exploited therapeutically (Figure)?

Recent work continues to highlight the attractiveness of Pim-1 as a target to enhance the efficacy of CPCs and perhaps the regenerative capacity of the injured myocardium. What, then, is the most promising approach to translating these findings into something more therapeutically relevant? The current article provides ample evidence that Pim-1 should be pursued and warrants further investigation into the mechanism responsible for the enhanced proliferation, differentiation, and self-renewal of CPCs elicited by this cardioprotective kinase. Specifically, what are the downstream targets?
necessary to mediate these beneficial outcomes? Is it possible to directly stimulate Pim-1 expression? Could Pim-1 therapy be combined with additional interventions, such as IGF-1, to further enhance the regenerative potential of CPCs? It is certainly possible to imagine a course of treatment involving the isolation of CPCs from a patient, providing these CPCs with a “regenerative boost” through modulation of Pim-1 or other additional factors, and the subsequent readministration of these CPCs to the injured myocardium. A more complete understanding of the mechanism underlying the benefits of enhanced ACS, the identification of novel markers of CPCs with increased regenerative capacity, and more effective methods of isolating these true stem-like progenitor cell populations should further improve cell-based therapy for ischemic heart disease.

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Dominic P. Del Re and Junichi Sadoshima

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