Biased DNA Segregation and Cardiac Stem Cell Therapies

Richard P. Harvey, Shahragim Tajbakhsh

Ischemic heart disease is one of our greatest killers and the epidemic of heart failure in Western countries is now being replicated in developing nations. In the headlong search for new therapies that will stimulate replacement of cardiomyocytes and vasculature lost to injury, great hope is being placed on stem cell and regeneration therapies. In this edition of the Circulation Research, Kajstura and colleagues1 report that a minority of human cardiac stem cells (hCSCs), when they divide in vitro, undergo asymmetrical chromatid segregation (ACS), whereby the ancestral DNA strands of all of their chromosomes segregate to 1 of the 2 daughter cells, presumably a property of executive stem cells. Preparations of stem cells enriched in those undergoing ACS have a superior ability, when injected into the border zone of rats at the time of myocardial infarction, to expand, migrate into the infarct zone, preserve function, and prevent adverse remodeling.

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A fundamental property of stem cells is their ability to self-renew, and in doing so maintain stem cell character in at least 1 of the daughter cells of a stem cell division. If they also give rise to more differentiated cells for tissue repair, they can undergo asymmetrical cell division—that is, give rise to 1 stem cell and 1 cell destined to differentiate. A stem cell can also undergo symmetrical divisions, either to amplify the number of stem cells exponentially, or commit both daughters to the differentiation pool. Whether it divides asymmetrically or symmetrically is dependent on both the properties of the cell itself, and on cues from its niche environment. During asymmetrical cell divisions, cell fate determinants segregate to one of the poles of the cell. When the mitotic spindle orients so that the cleavage plane is orthogonal to the polarity axis, only 1 daughter cell receives the lineage determinants. Many of the molecular players of asymmetrical cell division have been elucidated over the last decade. There are in fact multiple mechanisms for the generation of alternative cell fates, but a general feature involves the asymmetrical cortical distribution and regulation of a kinase complex containing PDZ domain PAR proteins (PAR3/6) and an atypical protein kinase C (aPKC).2 The Notch signaling pathway can be involved in asymmetrical cell division, and its asymmetrical distribution and/or activation in one of the daughter cells is in some cases governed by the multifunctional adaptor protein Numb (Numb and Numb-like in mammals), where the asymmetrical localization of Numb and a bound effector protein, α-Adaptin, are central to the mechanism.3

In 1975, John Cairns proposed, based on observations of the way primary cells in culture retain labeled nucleotides for long periods without dilution, that in some stem cell divisions the oldest DNA strands of each chromosome were effectively immortal because they always segregated to only 1 of the 2 daughter cells, leading to the so-called immortal DNA strand hypothesis.3,4 This process, termed ACS by Kajstura and colleagues (also known as template, biased, or nonrandom DNA strand segregation), has been observed in a number of cell types, in the contemporary era aided by the use of more rigorous techniques including fluorescent chromophore-tagged or natural isotope nucleotides, live cell imaging, genetic stem cell tagging, chromosome orientation-fluorescence in situ hybridization, and careful staging of cell divisions and cell fate outcomes in the in vivo tissue context, as well as ex vivo.5–7 However, its presence in vivo remains controversial4 and thus documentation of its absence or presence requires the highest standards of experimentation. The attraction of asymmetrical chromatid segregation for stem cells is that it would provide a mechanism whereby they could be protected from accumulating mutations in their DNA due to errors during replication, thereby maintaining a more “pristine” genome. Although this has not been proven nor disproven, mechanisms such as nucleotide excision/repair and sister chromatid exchange compromise the notion and thus the immortal purity of the DNA. Another attractive hypothesis is that ACS has evolved as a distinct regulatory machinery for specifying distinct cell fates in daughter cells.4 Whatever the case, the discovery of ACS highlighted an entirely new mechanism in cell biology that broke the golden rule of random chromosome assortment in cells that divide asymmetrically and implies a form of asymmetrical cell division that may specify or accompany asymmetrical decisions of cell fate.

In a series of experiments, Kajstura and colleagues1 show that a minority of in vitro clones of human endogenous cKit+ cardiac stem cells, that have been characterized previously by these authors8 and which have recently entered clinical trials,9,10 show the properties of ACS. Around 12% of clones of hCSCs isolated from the hearts of young children showed ACS, and although this percent diminished with age, clones could still be identified in octogenarians. The use of pulse-chase protocols with single or multiple nucleotide analogues and microscopy to track newly synthesized DNA strands in

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From the Developmental and Stem Cell Biology Division (R.P.H.), Victor Chang Cardiac Research Institute, Darlinghurst, New South Wales, Australia; St. Vincent’s Clinical School (R.P.H.), The University of New South Wales, Kensington, Australia; and Laboratory of Stem Cells and Development (S.T.), Department of Developmental and Stem Cell Biology, CNRS URA 2578, Pasteur Institute, Paris, France.

Correspondence to Richard P. Harvey. Developmental and Stem Cell Biology Division, Victor Chang Cardiac Research Institute, Lowy Packer Building, 405 Liverpool Street, Darlinghurst NSW 2010, Australia. E-mail r.harvey@victorchang.edu.au


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sequential cell divisions (Figure) has allowed Kajstura and colleagues to claim a number of properties for ACS as it applied to hCSCs in vitro, namely 1) ACS is highly stable in isolated clones across many cell divisions without apparent cell commitment; 2) daughter cells receiving newly synthesized DNA also divide by ACS; 3) clones undergoing ACS proliferate faster than those undergoing symmetrical chromosome segregation (SCS); and 4) ACS clones partially preserve telomere length and show minimal expression of markers of senescence or DNA damage. Because previous studies reported that some but not all chromatids undergo ACS, it would be prudent to extend these studies to single chromosome resolution using chromosome orientation-fluorescence in situ hybridization, a technique that allows the precise direction of newly synthesized DNA to be detected on every chromosome, and thus to distinguish whether all chromosomes undergo ACS in all cell divisions.4,5,7 Furthermore, a major point of confusion in the field is discriminating between the “immortality” of DNA strands, which presumes obligate ACS and very long retention of nucleotide analogues in the experimental setting, from ACS that takes place but not in an obligate manner due to intervening SCS (old DNA strands are therefore never really “immortal”). However, the latter case would lead to dilution of nucleotide analogues over many cell divisions and this did not happen in the Kajstura study of hCSCs in vitro.

ACS can accompany asymmetrical segregation of cell fates through asymmetrical cell division,7 although too few examples are currently available to determine whether it must always do so. In the Kajstura et al study,1 ACS occurred without asymmetrical partitioning of α-Adaptin (which binds to Numb) at anaphase/telophase, suggesting that ACS might be uncoupled from asymmetrical segregation of cell fate determinants. No markers of differentiated lineages were detected in the cultures, supporting the notion that they have symmetrical cell fates. Because Numb is a multifunctional adaptor protein, a kinase target, and cargo of the asymmetry machinery, other markers such as PAR3/6 and aPKC, should be analyzed to validate this point. In addition, the culture conditions used in this study might favor symmetrical (amplifying) stem cell divisions for these hCSCs. Other conditions permissive for differentiation, such as lower growth factor or serum concentrations, might display both asymmetrical cell division and ACS. This raises an interesting issue: does ACS require the asymmetrical cell division machinery? The mechanism of sorting of old and new DNA strands in ACS is unknown and may rely more on recognition of old and new chromatin marks than asymmetrical cell division machinery. The seemingly robust ACS discovered by Kajstura and colleagues in hCSCs will be a good system in which to test this relationship. Indeed, they show dependence of ACS in hCSCs on the microtubule motor protein left-right dynein, which has been suggested in other studies to control left-right body asymmetry via a developmental mechanism involving ACS.11
Having defined a minority population of hCSCs undergoing ACS, Kajstura and colleagues asked whether this population had executive stem cell functions in a stem cell therapy protocol in rats. To enrich for cells that show the properties of ACS (referred to in the article as cell with “old DNA”) and those undergoing symmetrical chromatid segregation (cells with “new DNA”), 2 optical techniques were applied. The first technique relies on differential resonance energy transfer between fluorescent DNA dyes in the 2 populations. After labeling mass cultures of cKit+ hCSCs for several population doublings with the nucleotide analog BrdU to achieve labeling of all cells (and virtually all DNA strands), they were allowed to divide without BrdU (chase), then stained with the nonintercalating DNA dye propidium iodide and the intercalating dye TO-PRO3, whose fluorescence enhancement is emitted if it intercalates in the vicinity of incorporated BrdU. In this complex but elegant protocol, a proportion of cells undergoing ACS (≈4% of the population) are not labeled by BrdU (see the Figure). All other cells carry BrdU and, when irradiated at the absorption wavelength of propidium iodide, show enhanced fluorescence energy transfer between propidium iodide and TO-PRO3 to give them a higher TO-PRO3 fluorescence signal than cells undergoing ACS, thereby allowing separation of the respective populations by flow cytometry.

In a second protocol, cells were labeled with BrdU and subjected to a chase period without BrdU, as above. Using the photosensitivity of cells carrying BrdU, cultures were irradiated briefly with UV-light and cells bearing BrdU were eliminated by apoptosis. Surviving cells had a grossly normal karyotype and a population doubling time and telomere length consistent with enrichment for cells undergoing ACS. These protocols, although useful, might not be amenable to human therapies because of the use of toxic nucleotide analogues and DNA-damaging irradiation.

Populations enriched for hCSCs undergoing ACS (carrying old DNA) were transduced with a lentivirus expressing green fluorescent protein (EGFP) to track their descendants and then injected into the border zones of immunosuppressed rats that had undergone surgery to induce acute myocardial infarction (MI). Control MI rats were injected with a cell population enriched for cKit+ cells predominantly undergoing Asymmetrical cell division (ACS; referred to in the article as cell with “old DNA”) and TO-PRO3 fluorescence signal than cells undergoing ACS, thereby allowing separation of the respective populations by flow cytometry.

In a final touch, the authors attempted to show that a viable hCSC niche could be reestablished in transplanted MI hearts. They noted the presence of small green-fluorescent and cKit+ cells in the interstitium of restored myocardium of MI rats and after isolation and transplantation of these cells without further manipulation, myocardium and vessels were again restored in secondary recipients. The ability to reconstitute an organ system such as blood or mammary gland in a serial fashion using single stem cells establishes a formidable experimental platform for stem cell science and is the envy of all stem cell biologists working in less tractable systems. Next best is to show serial transplantation of a stem cell phenotype within a population of cells, which indirectly suggests preservation of stem-like character in cells that either fail to differentiate and/or become established in a new stem cell niche. Although the experiments of Kajstura and colleagues are a promising start, they will need to examine ACS or otherwise characterize the recovered cells, which may predominantly be committed progenitors.

Overall, this body of work reports on an in vitro analysis of cardiac-derived stem-like cells that have the intriguing property of nucleotide label distribution compatible with ACS. It adds to observations that cKit+ cells predominantly undergo asymmetrical cell division as judged by asymmetrical partitioning of Numb and α-Adaptin in cardiac tissue sections. ACS appears highly stable in hCSCs over many cell divisions and may provide a robust platform for investigating the mechanistic links to asymmetrical cell fate decisions. Finally, the finding that hCSC clones showing ACS deliver superior outcome when used in transplantation assays, which may be due to their higher division rate or other properties such as increased survival, opens the way to new approaches in humans if protocols can be found for enriching for ACS without the use of cytotoxic treatments. The long-term outcomes of cKit+ hCSC clinical trials are eagerly awaited. The cardiac cell therapy field is at an interesting nexus—although it appears that stem cells can deliver short- and long-term salubrious effects to patients via paracrine mechanisms, only some animal disease models studied have shown significant deployment of stem cells and some researchers remain circumspect about this possibility in humans. The studies of Kajstura and colleagues reported here and previously suggest that the right stem cell populations delivered in the right way might have a transformative outcome.

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None.

**References**


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