To Seek the Holy Grail of Cardiac Progenitor Cells

An Opera in Four Acts

A.J. Marian

Discoveries in cardiovascular stem cell biology, myocyte regeneration, and cell transplantation have enormous clinical potentials, yet the field is embroiled in amusing controversies.[1–3] The existence of cardiac myocyte progenitor cells (CPCs) in the adult heart, let alone the identity of the genuine CPCs, has been debated and so has the relevance of the primary candidate, namely the resident cardiac progenitor cells expressing the KIT antigen (KIT^{pos} cells), to cardiac myocyte generation.[1,2,4] Similarly, the choice of the cells, delivery approach, and efficacy of cell transplantation in improving cardiovascular outcomes have remained unsettled as has been the elusive nature of the paracrine factors that are presumably responsible for the improvement in cardiac function post-cell transplantation. Consequently, given these ambiguities, basic and clinical investigators are in the quest to seek the holy grail of CPCs; cells that possess genuine capacities to survive, thrive, differentiate to working myocytes, properly couple with the neighboring resident myocytes; electrically and mechanically, enhance cardiac function, and improve the clinical outcomes, without causing serious adverse events. Similarly, there is an intense focus on defining the responsible mechanisms and identifying and characterizing the elusive paracrine factors.

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Tale of Hippopotamus (Act I)

The Hippo pathway, first discovered in *Drosophila* and aptly named after hippopotamus because of its key role in determining organ size, is a contact-dependent pathway that regulates cell growth, survival, proliferation, and differentiation.[5,6] The pathway comprises a series of upstream molecules that are linked to cell junctions and are sensitive to cell–cell contact. The upstream molecules are also responsive to mechanical properties of cell environment and cell cytoskeleton, particularly actin filament organization. On sensing the stimuli, the upstream Hippo molecules activate a cascade of multifunctional serine–threonine kinases, which subsequently phosphorylate YAP1 (yes-associated protein 1), leading to its nuclear exclusion and hence, inactivation. YAP1 along with its paralogue transcriptional coactivator WW domain containing transcription regulator 1 (WWTR1, also known as TAZ) binds to TEAD (TEA domain transcription factor 1) family of transcription factors (and others) and stimulates expression of a large number of genes involved in cell growth, proliferation, and resistance to apoptosis. Therefore, the downstream effects of activation of the Hippo cascade is inactivation of YAP1-WWTR1-TEAD transcriptional machinery and suppression of gene expression. Conversely, inactivation of the upstream Hippo molecules results in increased gene expression through YAP1-WWTR1-TEAD transcriptional complex. The latter results in a faster cell growth and proliferation, and decreased apoptosis, whereas the former exerts the opposite effects.

Castle of Camelot (Act II)

Cell junctions are the hubs for several upstream components of the Hippo pathway and consequently, its main regulators.[5] However, in contrast to most conventional signaling pathways that originate at the cell cytoplasmic membrane and have specific sets of cell surface receptors, the Hippo pathway is not known to have an exclusive set of receptors at the cell membrane. Instead, the pathway is regulated through a diverse array of mechanisms by other biological networks that incorporate the key constituents of the Hippo pathway into their molecular webs. Nevertheless, the vast majority of the mechanisms that regulate the Hippo pathways are eventually linked to cell junctions. The prominent role of cell junctions in regulating the Hippo pathways is exemplified in arrhythmogenic right ventricular cardiomyopathy, which is primarily a disease of cell junction proteins.[7] The Hippo pathway is markedly activated in arrhythmogenic right ventricular cardiomyopathy and contributes to the pathogenesis of its phenotype.[8] Likewise, mechanisms that affect cell cytoskeleton and F-actin organization, such as the GPCR (G-protein–coupled receptor) signaling, also regulate gene expression through the YAP1-WWTR1-TEAD transcriptional complex.[9]

Knights of the Round Table (Act III)

ATP—a common energy currency of the cell—and other purine and pyrimidine nucleotides, when released into the extracellular space on cell injury, impart effects through activation of over a diverse but cell-type specific set of a dozen transmembrane purinergic receptors. The receptors, with the exception of the subfamily P2RX (purinergic type 2 receptors, subtype X), have typical features of the GPCRs. The P1R subfamily, whose ligand is adenosine, and a subgroup of the P2R family, namely P2RYs (purinergic type 2 receptors, subtype Y), which primarily responds to ATP, exhibit various levels of selectivity in coupling with the heterotrimeric G protein subunits. The diversity of the purinergic receptors along with their differential affinity for the G protein subunits, encoded by over a dozen

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genes, affords the system an enormous versatility in activating a multitude of downstream signaling pathways and hence, exerting wide-ranging biological effects. Accordingly, the purinergic receptor system has been implicated in platelet aggregation, inflammation, systemic arterial hypertension, atherosclerosis, cardiac contractility, hypertrophy, and heart failure.[10]

A particular feature of the P2RY signaling is coupling with the Gα subunits, including the Gα12/13 proteins, which are encoded by *GNA12* and *GNA13* genes, respectively. The coupling leads to activation of the Rho family of GTPases, including RHOA (Ras homolog family member A), which is a major regulator of intracellular actin dynamics. Cytoskeleton actin, as discussed earlier, is a major and well-established regulator of the Hippo pathway. Thus, the Hippo pathway might function downstream to the GPCRs, including a subset of the purinergic receptors that couple with certain G protein subunits.[9]

Quest for Holy Grail (Act IV)

The preceding prelude and the 3 acts provide 4 lines of impetus for seeking the Holy Grail of CPCs. The prelude illustrated the shortcomings of the current CPCs. Act I described the Hippo pathway as a prosurvival and progrowth pathway. Act 2 identified junctions as the major signaling hubs and the command center of the Hippo pathway. Act 3 identified the P2RYs as multitalented partners of the G protein subunits, involved in various biological functions, including possibly regulating the Hippo pathway. The assemblage enabled the group led by Heartmanforever to invigorate the human KIT^{pos} cells by activating the P2RYs for a superior biological performance.[11] The investigators categorize the human KIT^{pos} cells into slow- and fast-growing CPCs and relate the growth rate to differential expressions of several P2RYs. Accordingly, levels of *P2ry1*, *P2ry2*, and *P2ry14* transcripts were lower in the slow- as compared with fast-growing CPCs. The biological significance of the differential transcript levels was illustrated by overexpressing P2RY2 in the human KIT^{pos} cells and by adding P2YR agonist UTP to the culture media and showing enhanced proliferation and migration of the stimulated cells. Mechanistically, the authors link the enhanced biological properties of the KIT^{pos} cells to activation of YAP1—the transcriptional coeffector of the Hippo pathway—by UTP.

Epilogue

The findings, reporting enhancing proliferation and migration of the KIT^{pos} CPCs in vitro, on activation of the P2RYs, are prelude to determining additional biological properties and clinical applications of these energized CPCs. Evidence to show enhanced differentiation of the KIT^{pos} cells to cardiac myocytes or other cardiac lineages on treatment with P2RY agonists will raise the interest in potential clinical utilities of these cells. This would be most welcome particularly, considering the recent data seriously challenging the role of KIT^{pos} cells in cardiac myocyte regeneration.[2] Likewise, in vivo evidence for increased retention, survival, and coupling of the UTP-stimulated CPCs with the native myocytes in the myocardium would further raise the interest in these cells, as would be the absence of adverse effects. Furthermore, the discovery of novel or potent paracrine factors, expressed by the ATP/UTP-energized as compared with nonstimulated KIT^{pos} CPCs, would lend further credibility to their in vivo applications. Moreover, delineating the specific mechanisms by which stimulation of the P2RYs activates YAP1 would expand the opportunities for targeting the Hippo pathways to enhance regeneration of cardiac myocytes and so would extension of the findings to other GPCRs.

The quest has just begun. It traverses through the treacherous land that hosts the none shall pass bridge, guarded by the Black Knight; the gate keeper of the bridge of death to whom 3 questions must be correctly answered or will be cast into the Gorge of Eternal Peril; the wicked and naughty Zoot of the Castle of Anthrax, whose sole quest is to deceive; the fire-loving Tim, the Enchanter, and finally, the killer rabbit of Caerbannog, who is the most ferocious beast of all. Nevertheless, the quest to seek the Holy Grail must continue. The Grail will reveal itself to those with the purity of Sir Galahad.

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Disclosures

None.

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