Cardiac Remodeling and Repair

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Repairing cardiac damage and restoring heart function includes cell-based, non-cellular, induced adult cardiomyocyte proliferation and manipulation of cardiac remodeling. Though there has been significant success in delineating the mechanism of cardiac injury and protection against acute ischemic injury, an efficient therapeutic intervention is still unavailable.

cardiac repair regeneration MicroRNA cell therapy

1. Introduction

Cardiovascular diseases continue to be the leading cause of morbidity and mortality in the United States and worldwide [1]. Despite recent studies showing that neonatal murine and porcine hearts are able to regenerate via proliferating cardiomyocytes within the first few days after birth, adult mammalian cardiomyocytes (ACMs) lack meaningful endogenous proliferative potential. This lack of proliferation results in pathological repair mechanisms and fibrotic scarring after cardiac injury [2][3][4]. Various approaches have been tested to repair cardiac damage and restore heart function in the past few decades. These strategies include cell-based, non-cellular, induced adult cardiomyocyte proliferation and manipulation of cardiac remodeling. Though there has been significant success in delineating the mechanism of cardiac injury and protection against acute ischemic injury, an efficient therapeutic intervention is still unavailable. Therefore, it is of great importance to reanalyze the research development, interpret common/global outcomes, and identify the potential pitfalls in developing future therapeutic interventions.

2. Cell-Based Approaches to Cardiac Repair

Bone marrow cells (BMCs) and embryonic stem cells (ESCs) also appeared to be promising alternatives to skeletal muscle myoblasts (SMs) for cardiac repair after Myocardial infarction (MI) with similar approaches. Like SMs, BMCs and ESCs cell transplants show improved cardiac function and decreased cardiac fibrosis in rodent models post-MI [5][6][7]. Similar to SMs, these cells are also easy to obtain and show the potential to differentiate into cardiomyocytes. However, these cells require pre-differentiation into mature cardiomyocytes prior to transplantation into an infarcted heart, as undifferentiated BMC or ESC carry the risk of developing teratomas after cell transplantation [8][9][10]. Unfortunately, to date, the lack of a standardized protocol to obtain a pure population of matured cardiac cells from BMCs or ESCs limits their therapeutic success [11]. Lastly, the risk of immunologic rejection because of genomic instability provides further concern for the utility of these cells [12].

More recent studies have brought attention to the use of MSCs and iPSCs that promise a greater cell fate plasticity and cardiomyocyte potential during cardiac repair post-MI, where cell transplants resulted in improved cardiac function in the injured heart in preclinical studies [13][14][15]. Of particular advantage in these studies was the ease of obtaining MSCs from various tissue types, albeit with varying efficacy [16]. Moreover, both MSC and iPSC have the potential of self-renewal and low immunogenicity, thus, are suitable for autologous transplantation [17][18][19]. Additionally, these cells showed an enhanced ability to differentiate into various cell types, including cardiomyocytes, and exhibit an ability to integrate into the host myocardium [20][21][22][23]. Despite being easy to obtain and propagate, complete differentiation of MSCs and iPSCs into cardiomyocytes continues to pose the most significant challenge of utilizing these cells therapeutically [24]. In fact, cardiomyocytes derived from MSCs and iPSCs show a variety of maturity and transcriptomic states similar to neonatal cardiomyocytes, which results in a heterogeneous population of undifferentiated cells [8][25]. Similar to BMCs and ESCs, MSCs and iPSCs also pose the risk of teratoma formation, limiting these cells' therapeutic potential [26].

Alternative approaches such as using a heterogeneous mix of cardio-sphere-derived cells (c-kit+, CD105+, and CD90+) have been the basis for a randomized phase one clinical trial showing a modest improvement with reduced scar size, and increased viability of myocardium; however, in terms of cardiac function, neither end-diastolic volume, end-systolic volume, or LVEF showed major difference between groups by 6 months [27]. Another report identified a subpopulation of cardiac mesenchymal cells, categorized as c-kit expressing and slow adhering when cultured, that improved cardiac function with significant recovery of LVEF after MI [28]. Moreover, a repetitive administration of these cardiac progenitor cells showed a more significant cardioprotective effect and a restoration of cardiac function when compared to a single administration of an equal number of cumulative cells [29].

Moreover, the issues surrounding the stability and survivability of transplanted cells in a harsh ischemic and fibrotic myocardium are still the foremost limiting factor for the therapeutic success of cell-based approaches to repairing the heart. To this end, a number of studies have been performed with a primary focus to improve the intra-myocardial delivery and engraftment of cells. For example, a recent report successfully demonstrated enhanced engraftment of ESC-derived cardiomyocytes in the heart using an injectable nanomatrix gel [30]. Furthermore, improved survivability of ESC-derived cardiomyocytes upon encapsulation with nanomatrix gel resulted in reduced scar formation and retention of LVEF in treated animals compared to controls. Another study utilized platelet surface markers to label cardiac stem cells (CSC), enhancing their recruitment in the injured heart and improving retention of labeled CSC into the infarcted region. This resulted in a decreased infarct size and improved cardiac function [31]. Moreover, mediation of gene transfer to bone marrow-derived MSCs (BMMSCs) via chemically modified nanoparticle, molecularly organic-inorganic hybrid hollow mesoporous organosilica nanoparticle (HMON), led to improved survivability of these cells in the infarcted heart after transplantation, which improved cardiac function with a complete recovery of LVEF values, reduced cardiac fibrosis, and increased angiogenesis [32]. Although several small, non-randomized cell-based studies have been conducted with these approaches, yielding some satisfactory success, extensive randomized studies fail to replicate these effects. Thus, a search remains for the ultimate cell type or a sustained delivery method for complete cardiac repair and restoration of normal function post-MI.

3. Cell-Free Approaches for Cardiac Repair

Regardless of the rapid death of implanted cells in the injured myocardium, most studies show improved cardiac function and, vascularization, as well as reduced infarct size, which leads to the hypothesis that paracrine factors may have an important role in cardiac repair. It has been observed that all the progenitor and stem cells used in cell-based therapeutics release secretory factors that have a paracrine effect on the neighboring cells. Secretome analysis revealed that these exosomes are concentrated alongside pro-angiogenic, pro-survival, proliferative, and immunogenic factors [33][34][35][36][37][38][39]. Interestingly, the content and composition of the secretome are highly dependent on the parental cell type and the physiological or pathophysiological condition. For example, one study showed that GATA-4 overexpressing MSCs secreted paracrine factors, which augmented angiogenesis and improved cell survivability in the ischemic myocardium [38]. In contrast, another study showed that overexpression of the pro-angiogenic miR-126 in MSCs leads to secretomes enriched with pro-angiogenic and pro-survival factors. Further mechanistic analysis of miR-126 over-expressing cells revealed increased expression of Notch ligand Delta-like (Dll)-4 is believed to play a key role in improving angiogenesis and blood flow in the infarcted heart, resulting in improved cell survivability and cardiac function with significantly higher LVEF values in miR-126 over-expressing cell treatment group compared to MSC alone [40]. Similar results were obtained in animals treated with secretomes isolated from human adipose-derived stem cells post-MI [37].

As recent studies highlight, exosomes are on the rise as a therapeutic asset since they are a major constituent of the secretome and deliver most of its attributed protective features. These nano-sized vesicles are secreted by most cell types, including cardiomyocytes and iPSCs [41][42][43][44][45][46][47]. Exosomes were first studied for their role in the adaptive immune response [48]. Subsequently, they were observed to play a vital role in signal transduction, cell-to-cell communication, and other paracrine mechanisms [49][50][51]. Further scientific advancements utilized exosomes derived from different cell types to study their therapeutic significance in various disease conditions, including MI [52][53]. Exosomes have also been explored as a plausible tool to deliver small molecules, such as miRNA or therapeutic drugs, to areas of interest [54][55]. Given the limited success of developing efficient cell transplantation approaches, research focus was shifted to analyze the protective effects of paracrine factors secreted by engrafted cells into the infarcted heart. These studies revealed that most of the protective effects from cell-based therapies were indirect through secretomes or, more specifically, exosomes. Similar to secretomes, exosomes also exhibit the features of their parent cells, such as ESC-derived exosomes. Similar to ESCs, whose total miRNA content is comprised of about two-thirds of the miR-290 family, ESC-derived exosomes also express a high level of miR-290 [46]. Further investigation revealed that ESCs derived exosome-mediated delivery of miRNA-294 led to increased cell survival, angiogenesis, and proliferation, and thus improved cardiac repair and restoration of cardiac function post-MI [46].

Moreover, treatment with the exosomes isolated from cardiosphere-derived cells showed enhanced cardiac repair by improving cardiac function and decreasing infarct size in acute as well as chronic porcine MI models [56]. Furthermore, cardiac progenitor cells (CPCs) are known to regulate cardiac protection and repair. Notably, the exosomes derived from CPCs likewise increased cell survivability and proliferation of H9C2 cells through enhanced expression of Akt and activation of the Akt/mTOR pathway [57]. Interestingly, a pediatric study showed that

exosomes derived from neonatal CPCs improved cardiac function and repair, whereas exosomes derived from CPCs of older children required hypoxia preconditioning to exhibit cardioprotective benefits. These effects included increased angiogenesis and reduced fibrosis, resulting in improved cardiac function in the infarcted heart [58]. Moreover, human pericardial fluid-derived exosomes carry let-7b-5p, which targets the TGFBR1 gene. An exosome-mediated delivery of let-7b-5p to endothelial cells led to improved angiogenesis which could have a protective effect in cardiac repair [59]. Furthermore, CD34+ HSC-derived exosomes express a high level of pro-angiogenic miRNAs, including miR-126 and miR-130, which improved vascular formation in the injured heart [60]. A miRNA sequence analysis between MSC and MSC-derived exosomes showed similar miRNA profiling, demonstrating a mechanistic similarity between MSC and MSC-derived exosome-mediated cardiac repair. This emphasized a greater therapeutic importance of MSC-derived exosomes over MSCs themselves for cardiac repair [61]. Other studies further demonstrated that exosomes derived from the hypoxia preconditioned MSCs show greater expression of various miRNAs involved in improved cell survival, angiogenesis, and reduced fibrosis, leading to enhanced cardiac repair in comparison to normoxic MSCs-derived exosomes [62][63][64][65]. Further analysis revealed that preconditioning CSCs with MSC-derived exosomes also results in improved cell survival and angiogenic potency of CSCs [66].

Regardless of the significant advancement and exciting results in cell-free therapy for cardiac repair, none of these studies were able to demonstrate significant cardiac regeneration even in small animals [67][68][69]. In addition to that, there are various other basic questions and limitations which need to be answered before improved therapeutic success with these methods. One of the major limitations is that the extracellular vesicles or exosomes require a direct intra-myocardial injection to the heart. Additionally, it is known that exosomes exhibit the features of the parental cell type, thus, it would be imperative to ensure that an autologous exosome does not carry any functional limitation when isolated from old cells.

4. Induced Cardiomyocyte Proliferation for Cardiac Regeneration and Repair

The senescent nature of adult mammalian cardiomyocytes (ACM) restricts their ability to proliferate and regenerate or repair after cardiac injury. However, zebrafish and neonatal mice demonstrate a propensity to regenerate cardiac tissue following injury, mostly facilitated by the proliferation of pre-existing cardiomyocytes [3][70]. Irrespective of the limited success of cell-based cardiac repair approaches, it remains of interest to induce the proliferation of endogenous ACM as a novel therapeutic approach to repair cardiac injury in adult animals. Importantly, in the last decade, proliferation studies with mammalian ACMs sufficiently demonstrated the scope of ACM proliferation through external interventions [71][72]. Reports demonstrate that the oxidative phosphorylation-based energy metabolism of ACMs leads to increased oxidative stress, resulting in elevated DNA damage response and, ultimately cell cycle arrest [73].

Contrary to the adult mammalian heart, a comparatively hypoxic environment of the fetal mammalian heart predominantly utilizes glycolysis for energy production and has the capability of cardiomyocyte proliferation [74][75][76]. Moreover, neonatal mouse cardiomyocytes exhibit fetal-like features during the first week of the proliferative

window, whereas a hypoxic exposure elongates the proliferative window in postnatal mice [73]. Furthermore, one study observed that hypoxic exposure induced the proliferation of endogenous cardiomyocytes in adult mice, which improved left ventricular function, and enhanced cardiac repair after MI [77]. During the last decade, various genes and miRNAs have been identified and characterized for their roles in cardiac development as well as ACM proliferation. Genome-wide analysis revealed Fam64a as a novel regulator of the cell cycle in fetal cardiomyocytes under hypoxic conditions. However, the expression of Fam64a significantly decreased in the postnatal cardiomyocytes after its exposure to the oxygen-rich environment [78]. In addition, ERBB2 plays an important regulatory role in cardiomyocyte proliferation during both embryonic and neonatal stages. The constitutive expression of ERBB2 showed dedifferentiation, cardiomyocyte proliferation, and improved cardiac function after MI. Further mechanistic analyses demonstrated that ERBB2 mediated dedifferentiation and proliferation through ERK and GSK3β/ β-catenin signaling pathways [79]. TBx20 also plays an important role in cardiomyocyte proliferation and normal cardiac development through activation of multiple signaling pathways, which are also associated with ACM proliferation and cardiac repair after MI. These include PI3K/AKT pathways, which improve ACM proliferation and survival [80][81]; HIPPO/YAP pathways, which improve cardiac regeneration, contractility, cardiac function, and survivability [82][83][84]; and BMP/Smad pathways, which improve cardiac repair, and function [85]. TBx20 also inhibits the expression of cell cycle inhibitors such as p21 and Meis1 by direct binding. Thus, an over-expression of TBx20 in infarcted hearts improves cardiac repair by inducing ACM proliferation [86].

Genome-wide functional screening identified forty miRNAs with the potential to induce neonatal cardiomyocyte proliferation in mice as well as rats. Moreover, this screening identified miR-590 and miR-199a for their potential to induce cardiomyocyte cell cycle re-entry in both neonatal and adult mice or rats [87]. Similarly, miR-210 overexpression in adult cardiomyocytes promoted cell survival, proliferation of ACMs, reduced fibrosis, and increased angiogenesis [88].

References

1. Virani, S.S.; Alonso, A.; Aparicio, H.J.; Benjamin, E.J.; Bittencourt, M.S.; Callaway, C.W.; Carson, A.P.; Chamberlain, A.M.; Cheng, S.; Delling, F.N.; et al. Heart Disease and Stroke Statistics-2021 Update: A Report From the American Heart Association. Circulation 2021, 143, e254–e743.
2. Ye, L.; D'Agostino, G.; Loo, S.J.; Wang, C.X.; Su, L.P.; Tan, S.H.; Tee, G.Z.; Pua, C.J.; Pena, E.M.; Cheng, R.B.; et al. Early Regenerative Capacity in the Porcine Heart. Circulation 2018, 138, 2798–2808.
3. Porrello, E.R.; Mahmoud, A.I.; Simpson, E.; Hill, J.A.; Richardson, J.A.; Olson, E.N.; Sadek, H.A. Transient regenerative potential of the neonatal mouse heart. Science 2011, 331, 1078–1080.
4. Yan, H.; Rao, X.; Wang, R.; Zhu, S.; Liu, R.; Zheng, X. Cell Cycle Withdrawal Limit the Regenerative Potential of Neonatal Cardiomyocytes. Cardiovasc. Eng. Technol. 2021, 12, 475–484.

5. Kajstura, J.; Urbanek, K.; Perl, S.; Hosoda, T.; Zheng, H.; Ogórek, B.; Ferreira-Martins, J.; Goichberg, P.; Rondon-Clavo, C.; Sanada, F.; et al. Cardiomyogenesis in the adult human heart. Circ. Res. 2010, 107, 305–315.
6. Caspi, O.; Huber, I.; Kehat, I.; Habib, M.; Arbel, G.; Gepstein, A.; Yankelson, L.; Aronson, D.; Beyar, R.; Gepstein, L. Transplantation of human embryonic stem cell-derived cardiomyocytes improves myocardial performance in infarcted rat hearts. J. Am. Coll. Cardiol. 2007, 50, 1884–1893.
7. Ménard, C.; Hagège, A.A.; Agbulut, O.; Barro, M.; Morichetti, M.C.; Brasselet, C.; Bel, A.; Messas, E.; Bissery, A.; Bruneval, P.; et al. Transplantation of cardiac-committed mouse embryonic stem cells to infarcted sheep myocardium: A preclinical study. Lancet 2005, 366, 1005–1012.
8. Talkhabi, M.; Aghdami, N.; Baharvand, H. Human cardiomyocyte generation from pluripotent stem cells: A state-of-art. Life Sci. 2016, 145, 98–113.
9. Lee, A.S.; Tang, C.; Cao, F.; Xie, X.; van der Bogt, K.; Hwang, A.; Connolly, A.J.; Robbins, R.C.; Wu, J.C. Effects of cell number on teratoma formation by human embryonic stem cells. Cell Cycle 2009, 8, 2608–2612.
10. Lin, Q.; Fu, Q.; Zhang, Y.; Wang, H.; Liu, Z.; Zhou, J.; Duan, C.; Wang, Y.; Wu, K.; Wang, C. Tumourigenesis in the infarcted rat heart is eliminated through differentiation and enrichment of the transplanted embryonic stem cells. Eur. J. Heart Fail. 2010, 12, 1179–1185.
11. Dahlmann, J.; Awad, G.; Dolny, C.; Weinert, S.; Richter, K.; Fischer, K.D.; Munsch, T.; Leßmann, V.; Volleth, M.; Zenker, M.; et al. Generation of functional cardiomyocytes from rat embryonic and induced pluripotent stem cells using feeder-free expansion and differentiation in suspension culture. PLoS ONE 2018, 13, e0192652.
12. Singla, D.K. Embryonic stem cells in cardiac repair and regeneration. Antioxid. Redox. Signal. 2009, 11, 1857–1863.
13. Shabbir, A.; Zisa, D.; Suzuki, G.; Lee, T. Heart failure therapy mediated by the trophic activities of bone marrow mesenchymal stem cells: A noninvasive therapeutic regimen. Am. J. Physiol. Heart Circ. Physiol. 2009, 296, H1888–H1897.
14. Alfaro, M.P.; Pagni, M.; Vincent, A.; Atkinson, J.; Hill, M.F.; Cates, J.; Davidson, J.M.; Rottman, J.; Lee, E.; Young, P.P. The Wnt modulator sFRP2 enhances mesenchymal stem cell engraftment, granulation tissue formation and myocardial repair. Proc. Natl. Acad. Sci. USA 2008, 105, 18366–18371.
15. Zhu, W.; Zhao, M.; Mattapally, S.; Chen, S.; Zhang, J. CCND2 Overexpression Enhances the Regenerative Potency of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes: Remuscularization of Injured Ventricle. Circ. Res. 2018, 122, 88–96.

16. Collins, E.; Gu, F.; Qi, M.; Molano, I.; Ruiz, P.; Sun, L.; Gilkeson, G.S. Differential efficacy of human mesenchymal stem cells based on source of origin. J. Immunol. 2014, 193, 4381–4390.
17. Karantalis, V.; Schulman, I.H.; Balkan, W.; Hare, J.M. Allogeneic cell therapy: A new paradigm in therapeutics. Circ. Res. 2015, 116, 12–15.
18. Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007, 131, 861–872.
19. Yu, J.; Vodyanik, M.A.; Smuga-Otto, K.; Antosiewicz-Bourget, J.; Frane, J.L.; Tian, S.; Nie, J.; Jonsdottir, G.A.; Ruotti, V.; Stewart, R.; et al. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007, 318, 1917–1920.
20. Szaraz, P.; Gratch, Y.S.; Iqbal, F.; Librach, C.L. In Vitro Differentiation of Human Mesenchymal Stem Cells into Functional Cardiomyocyte-like Cells. J. Vis. Exp. 2017, 126, e55757.
21. Markmee, R.; Aungsuchawan, S.; Narakornsak, S.; Tancharoen, W.; Bumrungkit, K.; Pangchaidee, N.; Pothacharoen, P.; Puaninta, C. Differentiation of mesenchymal stem cells from human amniotic fluid to cardiomyocyte-like cells. Mol. Med. Rep. 2017, 16, 6068–6076.
22. Kempf, H.; Zweigerdt, R. Scalable Cardiac Differentiation of Pluripotent Stem Cells Using Specific Growth Factors and Small Molecules. Adv. Biochem. Eng. Biotechnol. 2018, 163, 39–69.
23. Guadix, J.A.; Orlova, V.V.; Giacomelli, E.; Bellin, M.; Ribeiro, M.C.; Mummery, C.L.; Pérez-Pomares, J.M.; Passier, R. Human Pluripotent Stem Cell Differentiation into Functional Epicardial Progenitor Cells. Stem Cell Rep. 2017, 9, 1754–1764.
24. Tu, C.; Chao, B.S.; Wu, J.C. Strategies for Improving the Maturity of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes. Circ. Res. 2018, 123, 512–514.
25. Veerman, C.C.; Kosmidis, G.; Mummery, C.L.; Casini, S.; Verkerk, A.O.; Bellin, M. Immaturity of human stem-cell-derived cardiomyocytes in culture: Fatal flaw or soluble problem? Stem Cells Dev. 2015, 24, 1035–1052.
26. Ahmed, R.P.; Ashraf, M.; Buccini, S.; Shujia, J.; Haider, H.K.h. Cardiac tumorigenic potential of induced pluripotent stem cells in an immunocompetent host with myocardial infarction. Regen. Med. 2011, 6, 171–178.
27. Makkar, R.R.; Smith, R.R.; Cheng, K.; Malliaras, K.; Thomson, L.E.; Berman, D.; Czer, L.S.; Marbán, L.; Mendizabal, A.; Johnston, P.V.; et al. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): A prospective, randomised phase 1 trial. Lancet 2012, 379, 895–904.
28. Wysoczynski, M.; Guo, Y.; Moore, J.B.; Muthusamy, S.; Li, Q.; Nasr, M.; Li, H.; Nong, Y.; Wu, W.; Tomlin, A.A.; et al. Myocardial Reparative Properties of Cardiac Mesenchymal Cells Isolated on

the Basis of Adherence. J. Am. Coll. Cardiol. 2017, 69, 1824–1838.

29. Tang, X.L.; Nakamura, S.; Li, Q.; Wysoczynski, M.; Gumpert, A.M.; Wu, W.J.; Hunt, G.; Stowers, H.; Ou, Q.; Bolli, R. Repeated Administrations of Cardiac Progenitor Cells Are Superior to a Single Administration of an Equivalent Cumulative Dose. J. Am. Heart Assoc. 2018, 7, e007400.
30. Ban, K.; Park, H.J.; Kim, S.; Andukuri, A.; Cho, K.W.; Hwang, J.W.; Cha, H.J.; Kim, S.Y.; Kim, W.S.; Jun, H.W.; et al. Cell therapy with embryonic stem cell-derived cardiomyocytes encapsulated in injectable nanomatrix gel enhances cell engraftment and promotes cardiac repair. ACS Nano 2014, 8, 10815–10825.
31. Tang, J.; Su, T.; Huang, K.; Dinh, P.U.; Wang, Z.; Vandergriff, A.; Hensley, M.T.; Cores, J.; Allen, T.; Li, T.; et al. Targeted repair of heart injury by stem cells fused with platelet nanovesicles. Nat. Biomed. Eng. 2018, 2, 17–26.
32. Zhu, K.; Wu, M.; Lai, H.; Guo, C.; Li, J.; Wang, Y.; Chen, Y.; Wang, C.; Shi, J. Nanoparticle-enhanced generation of gene-transfected mesenchymal stem cells for in vivo cardiac repair. Biomaterials 2016, 74, 188–199.
33. Nie, S.; Wang, X.; Sivakumaran, P.; Chong, M.M.W.; Liu, X.; Karnezis, T.; Bandara, N.; Takov, K.; Nowell, C.J.; Wilcox, S.; et al. Biologically active constituents of the secretome of human W8B2. Sci. Rep. 2018, 8, 1579.
34. Wagner, T.; Traxler, D.; Simader, E.; Beer, L.; Narzt, M.S.; Gruber, F.; Madlener, S.; Laggner, M.; Erb, M.; Vorstandlechner, V.; et al. Different pro-angiogenic potential of γ-irradiated PBMC-derived secretome and its subfractions. Sci. Rep. 2018, 8, 18016.
35. Korf-Klingebiel, M.; Reboll, M.R.; Klede, S.; Brod, T.; Pich, A.; Polten, F.; Napp, L.C.; Bauersachs, J.; Ganser, A.; Brinkmann, E.; et al. Myeloid-derived growth factor (C19orf10) mediates cardiac repair following myocardial infarction. Nat. Med. 2015, 21, 140–149.
36. Selvasandran, K.; Makhoul, G.; Jaiswal, P.K.; Jurakhan, R.; Li, L.; Ridwan, K.; Cecere, R. A Tumor Necrosis Factor-α and Hypoxia-Induced Secretome Therapy for Myocardial Repair. Ann. Thorac. Surg. 2018, 105, 715–723.
37. Waters, R.; Alam, P.; Pacelli, S.; Chakravarti, A.R.; Ahmed, R.P.H.; Paul, A. Stem cell-inspired secretome-rich injectable hydrogel to repair injured cardiac tissue. Acta Biomater. 2018, 69, 95–106.
38. Li, H.; Zuo, S.; He, Z.; Yang, Y.; Pasha, Z.; Wang, Y.; Xu, M. Paracrine factors released by GATA-4 overexpressed mesenchymal stem cells increase angiogenesis and cell survival. Am. J. Physiol. Heart Circ. Physiol. 2010, 299, H1772–H1781.
39. Kishore, R.; Khan, M. More Than Tiny Sacks: Stem Cell Exosomes as Cell-Free Modality for Cardiac Repair. Circ. Res. 2016, 118, 330–343.

40. Huang, F.; Zhu, X.; Hu, X.Q.; Fang, Z.F.; Tang, L.; Lu, X.L.; Zhou, S.H. Mesenchymal stem cells modified with miR-126 release angiogenic factors and activate Notch ligand Delta-like-4, enhancing ischemic angiogenesis and cell survival. Int. J. Mol. Med. 2013, 31, 484–492.
41. Harding, C.; Stahl, P. Transferrin recycling in reticulocytes: pH and iron are important determinants of ligand binding and processing. Biochem. Biophys. Res. Commun. 1983, 113, 650–658.
42. Pan, B.T.; Johnstone, R.M. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: Selective externalization of the receptor. Cell 1983, 33, 967–978.
43. Trams, E.G.; Lauter, C.J.; Salem, N.; Heine, U. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. Biochim. Biophys. Acta 1981, 645, 63–70.
44. Kowal, J.; Arras, G.; Colombo, M.; Jouve, M.; Morath, J.P.; Primdal-Bengtson, B.; Dingli, F.; Loew, D.; Tkach, M.; Théry, C. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. Proc. Natl. Acad. Sci. USA 2016, 113, E968–E977.
45. Vrijsen, K.R.; Sluijter, J.P.; Schuchardt, M.W.; van Balkom, B.W.; Noort, W.A.; Chamuleau, S.A.; Doevendans, P.A. Cardiomyocyte progenitor cell-derived exosomes stimulate migration of endothelial cells. J. Cell. Mol. Med. 2010, 14, 1064–1070.
46. Khan, M.; Nickoloff, E.; Abramova, T.; Johnson, J.; Verma, S.K.; Krishnamurthy, P.; Mackie, A.R.; Vaughan, E.; Garikipati, V.N.; Benedict, C.; et al. Embryonic stem cell-derived exosomes promote endogenous repair mechanisms and enhance cardiac function following myocardial infarction. Circ. Res. 2015, 117, 52–64.
47. Singla, D.K. Stem cells and exosomes in cardiac repair. Curr. Opin. Pharmacol. 2016, 27, 19–23.
48. Harding, C.V.; Collins, D.S.; Slot, J.W.; Geuze, H.J.; Unanue, E.R. Liposome-encapsulated antigens are processed in lysosomes, recycled, and presented to T cells. Cell 1991, 64, 393–401.
49. Corrado, C.; Raimondo, S.; Chiesi, A.; Ciccia, F.; De Leo, G.; Alessandro, R. Exosomes as intercellular signaling organelles involved in health and disease: Basic science and clinical applications. Int. J. Mol. Sci. 2013, 14, 5338–5366.
50. Su, S.A.; Xie, Y.; Fu, Z.; Wang, Y.; Wang, J.A.; Xiang, M. Emerging role of exosome-mediated intercellular communication in vascular remodeling. Oncotarget 2017, 8, 25700–25712.
51. Yellon, D.M.; Davidson, S.M. Exosomes: Nanoparticles involved in cardioprotection? Circ. Res. 2014, 114, 325–332.
52. Emanueli, C.; Shearn, A.I.; Angelini, G.D.; Sahoo, S. Exosomes and exosomal miRNAs in cardiovascular protection and repair. Vascul. Pharmacol. 2015, 71, 24–30.

53. Emanueli, C.; Shearn, A.I.; Laftah, A.; Fiorentino, F.; Reeves, B.C.; Beltrami, C.; Mumford, A.; Clayton, A.; Gurney, M.; Shantikumar, S.; et al. Coronary Artery-Bypass-Graft Surgery Increases the Plasma Concentration of Exosomes Carrying a Cargo of Cardiac MicroRNAs: An Example of Exosome Trafficking Out of the Human Heart with Potential for Cardiac Biomarker Discovery. PLoS ONE 2016, 11, e0154274.
54. Li, S.P.; Lin, Z.X.; Jiang, X.Y.; Yu, X.Y. Exosomal cargo-loading and synthetic exosome-mimics as potential therapeutic tools. Acta Pharmacol. Sin. 2018, 39, 542–551.
55. Stranford, D.M.; Leonard, J.N. Delivery of Biomolecules via Extracellular Vesicles: A Budding Therapeutic Strategy. Adv. Genet. 2017, 98, 155–175.
56. Gallet, R.; Dawkins, J.; Valle, J.; Simsolo, E.; de Couto, G.; Middleton, R.; Tseliou, E.; Luthringer, D.; Kreke, M.; Smith, R.R.; et al. Exosomes secreted by cardiosphere-derived cells reduce scarring, attenuate adverse remodelling, and improve function in acute and chronic porcine myocardial infarction. Eur. Heart J. 2017, 38, 201–211.
57. Li, S.; Jiang, J.; Yang, Z.; Li, Z.; Ma, X.; Li, X. Cardiac progenitor cell-derived exosomes promote H9C2 cell growth via Akt/mTOR activation. Int. J. Mol. Med. 2018, 42, 1517–1525.
58. Agarwal, U.; George, A.; Bhutani, S.; Ghosh-Choudhary, S.; Maxwell, J.T.; Brown, M.E.; Mehta, Y.; Platt, M.O.; Liang, Y.; Sahoo, S.; et al. Experimental, Systems, and Computational Approaches to Understanding the MicroRNA-Mediated Reparative Potential of Cardiac Progenitor Cell-Derived Exosomes From Pediatric Patients. Circ. Res. 2017, 120, 701–712.
59. Beltrami, C.; Besnier, M.; Shantikumar, S.; Shearn, A.I.; Rajakaruna, C.; Laftah, A.; Sessa, F.; Spinetti, G.; Petretto, E.; Angelini, G.D.; et al. Human Pericardial Fluid Contains Exosomes Enriched with Cardiovascular-Expressed MicroRNAs and Promotes Therapeutic Angiogenesis. Mol. Ther. 2017, 25, 679–693.
60. Sahoo, S.; Klychko, E.; Thorne, T.; Misener, S.; Schultz, K.M.; Millay, M.; Ito, A.; Liu, T.; Kamide, C.; Agrawal, H.; et al. Exosomes from human CD34(+) stem cells mediate their proangiogenic paracrine activity. Circ. Res. 2011, 109, 724–728.
61. Shao, L.; Zhang, Y.; Lan, B.; Wang, J.; Zhang, Z.; Zhang, L.; Xiao, P.; Meng, Q.; Geng, Y.J.; Yu, X.Y.; et al. MiRNA-Sequence Indicates That Mesenchymal Stem Cells and Exosomes Have Similar Mechanism to Enhance Cardiac Repair. Biomed Res. Int. 2017, 2017, 4150705.
62. Zhu, J.; Lu, K.; Zhang, N.; Zhao, Y.; Ma, Q.; Shen, J.; Lin, Y.; Xiang, P.; Tang, Y.; Hu, X.; et al. Myocardial reparative functions of exosomes from mesenchymal stem cells are enhanced by hypoxia treatment of the cells via transferring microRNA-210 in an nSMase2-dependent way. Artif. Cells Nanomed. Biotechnol. 2018, 46, 1659–1670.
63. Feng, Y.; Huang, W.; Wani, M.; Yu, X.; Ashraf, M. Ischemic preconditioning potentiates the protective effect of stem cells through secretion of exosomes by targeting Mecp2 via miR-22.

PLoS ONE 2014, 9, e88685.

64. Zhu, L.P.; Tian, T.; Wang, J.Y.; He, J.N.; Chen, T.; Pan, M.; Xu, L.; Zhang, H.X.; Qiu, X.T.; Li, C.C.; et al. Hypoxia-elicited mesenchymal stem cell-derived exosomes facilitates cardiac repair through miR-125b-mediated prevention of cell death in myocardial infarction. Theranostics 2018, 8, 6163–6177.
65. Gonzalez-King, H.; García, N.A.; Ontoria-Oviedo, I.; Ciria, M.; Montero, J.A.; Sepúlveda, P. Hypoxia Inducible Factor-1α Potentiates Jagged 1-Mediated Angiogenesis by Mesenchymal Stem Cell-Derived Exosomes. Stem Cells 2017, 35, 1747–1759.
66. Zhang, Z.; Yang, J.; Yan, W.; Li, Y.; Shen, Z.; Asahara, T. Pretreatment of Cardiac Stem Cells with Exosomes Derived From Mesenchymal Stem Cells Enhances Myocardial Repair. J. Am. Heart Assoc. 2016, 5, e002856.
67. Vagnozzi, R.J.; Molkentin, J.D.; Houser, S.R. New Myocyte Formation in the Adult Heart: Endogenous Sources and Therapeutic Implications. Circ. Res. 2018, 123, 159–176.
68. Lerman, D.A.; Alotti, N.; Ume, K.L.; Péault, B. Cardiac Repair and Regeneration: The Value of Cell Therapies. Eur. Cardiol. 2016, 11, 43–48.
69. Van Berlo, J.H.; Molkentin, J.D. An emerging consensus on cardiac regeneration. Nat. Med. 2014, 20, 1386–1393.
70. Poss, K.D.; Wilson, L.G.; Keating, M.T. Heart regeneration in zebrafish. Science 2002, 298, 2188–2190.
71. Bergmann, O.; Bhardwaj, R.D.; Bernard, S.; Zdunek, S.; Barnabé-Heider, F.; Walsh, S.; Zupicich, J.; Alkass, K.; Buchholz, B.A.; Druid, H.; et al. Evidence for cardiomyocyte renewal in humans. Science 2009, 324, 98–102.
72. Bicknell, K.A.; Coxon, C.H.; Brooks, G. Can the cardiomyocyte cell cycle be reprogrammed? J. Mol. Cell. Cardiol. 2007, 42, 706–721.
73. Puente, B.N.; Kimura, W.; Muralidhar, S.A.; Moon, J.; Amatruda, J.F.; Phelps, K.L.; Grinsfelder, D.; Rothermel, B.A.; Chen, R.; Garcia, J.A.; et al. The oxygen-rich postnatal environment induces cardiomyocyte cell-cycle arrest through DNA damage response. Cell 2014, 157, 565–579.
74. Kimura, W.; Xiao, F.; Canseco, D.C.; Muralidhar, S.; Thet, S.; Zhang, H.M.; Abderrahman, Y.; Chen, R.; Garcia, J.A.; Shelton, J.M.; et al. Hypoxia fate mapping identifies cycling cardiomyocytes in the adult heart. Nature 2015, 523, 226–230.
75. Simsek, T.; Kocabas, F.; Zheng, J.; Deberardinis, R.J.; Mahmoud, A.I.; Olson, E.N.; Schneider, J.W.; Zhang, C.C.; Sadek, H.A. The distinct metabolic profile of hematopoietic stem cells reflects their location in a hypoxic niche. Cell Stem Cell 2010, 7, 380–390.

76. Kimura, W.; Muralidhar, S.; Canseco, D.C.; Puente, B.; Zhang, C.C.; Xiao, F.; Abderrahman, Y.H.; Sadek, H.A. Redox signaling in cardiac renewal. Antioxid. Redox. Signal. 2014, 21, 1660–1673.
77. Nakada, Y.; Canseco, D.C.; Thet, S.; Abdisalaam, S.; Asaithamby, A.; Santos, C.X.; Shah, A.M.; Zhang, H.; Faber, J.E.; Kinter, M.T.; et al. Hypoxia induces heart regeneration in adult mice. Nature 2017, 541, 222–227.
78. Hashimoto, K.; Kodama, A.; Honda, T.; Hanashima, A.; Ujihara, Y.; Murayama, T.; Nishimatsu, S.I.; Mohri, S. Fam64a is a novel cell cycle promoter of hypoxic fetal cardiomyocytes in mice. Sci. Rep. 2017, 7, 4486.
79. D'Uva, G.; Aharonov, A.; Lauriola, M.; Kain, D.; Yahalom-Ronen, Y.; Carvalho, S.; Weisinger, K.; Bassat, E.; Rajchman, D.; Yifa, O.; et al. ERBB2 triggers mammalian heart regeneration by promoting cardiomyocyte dedifferentiation and proliferation. Nat. Cell Biol. 2015, 17, 627–638.
80. Bersell, K.; Arab, S.; Haring, B.; Kühn, B. Neuregulin1/ErbB4 signaling induces cardiomyocyte proliferation and repair of heart injury. Cell 2009, 138, 257–270.
81. Lin, Z.; Zhou, P.; von Gise, A.; Gu, F.; Ma, Q.; Chen, J.; Guo, H.; van Gorp, P.R.; Wang, D.Z.; Pu, W.T. Pi3kcb links Hippo-YAP and PI3K-AKT signaling pathways to promote cardiomyocyte proliferation and survival. Circ. Res. 2015, 116, 35–45.
82. Xin, M.; Kim, Y.; Sutherland, L.B.; Murakami, M.; Qi, X.; McAnally, J.; Porrello, E.R.; Mahmoud, A.I.; Tan, W.; Shelton, J.M.; et al. Hippo pathway effector Yap promotes cardiac regeneration. Proc. Natl. Acad. Sci. USA 2013, 110, 13839–13844.
83. Lin, Z.; von Gise, A.; Zhou, P.; Gu, F.; Ma, Q.; Jiang, J.; Yau, A.L.; Buck, J.N.; Gouin, K.A.; van Gorp, P.R.; et al. Cardiac-specific YAP activation improves cardiac function and survival in an experimental murine MI model. Circ. Res. 2014, 115, 354–363.
84. Heallen, T.; Morikawa, Y.; Leach, J.; Tao, G.; Willerson, J.T.; Johnson, R.L.; Martin, J.F. Hippo signaling impedes adult heart regeneration. Development 2013, 140, 4683–4690.
85. Sun, L.; Yu, J.; Qi, S.; Hao, Y.; Liu, Y.; Li, Z. Bone morphogenetic protein-10 induces cardiomyocyte proliferation and improves cardiac function after myocardial infarction. J. Cell. Biochem. 2014, 115, 1868–1876.
86. Xiang, F.L.; Guo, M.; Yutzey, K.E. Overexpression of Tbx20 in Adult Cardiomyocytes Promotes Proliferation and Improves Cardiac Function After Myocardial Infarction. Circulation 2016, 133, 1081–1092.
87. Eulalio, A.; Mano, M.; Dal Ferro, M.; Zentilin, L.; Sinagra, G.; Zacchigna, S.; Giacca, M. Functional screening identifies miRNAs inducing cardiac regeneration. Nature 2012, 492, 376–381.
88. Arif, M.; Pandey, R.; Alam, P.; Jiang, S.; Sadayappan, S.; Paul, A.; Ahmed, R.P.H. MicroRNA-210-mediated proliferation, survival, and angiogenesis promote cardiac repair post myocardial

infarction in rodents. J. Mol. Med. 2017, 95, 1369–1385.