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A chemical approach to myocardial protection and regeneration

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The possibility of generating induced pluripotent stem cells from mouse embryonic fibroblasts and human adult fibroblasts has introduced new perspectives for possible therapeutic strategies to repair damaged hearts. However, obtaining large numbers of adult stem cells is still an ongoing challenge, and the safety of genetic reprogramming with lenti- or retro-viruses has several drawbacks not easy to be addressed. Furthermore, the majority of adult stem cell-based clinical trials for heart regeneration have had generally poor and controversial results. Nonetheless, it is now clear that the injected cells activate the growth and differentiation of progenitor cells that are already present in the heart. This is achieved by the release of signalling factors and/or exosomes carrying them. Along this line, chemistry may play a major role in developing new strategies for activating resident stem cells to regenerate the heart. In particular, this review focuses on small molecule approaches for cell reprogramming, cell differentiation, and activation of cell protection.

Introduction

Myocardial damage often results in chronic failure due to loss and insufficient regeneration of cardiomyocytes.[1] This has prompted efforts to devise cardiomyocyte replacement therapies mainly by cell transplantation or by the promotion of endogenous regenerative processes.[2] Among others, stem cell-based approaches and, more recently, cell reprogramming[3] have generated an enormous attention as they promised to have a direct therapeutic application.

In particular, the generation of induced pluripotent stem cells (iPSCs) from mouse embryonic fibroblasts in 2006[4,5] and, shortly after, from human adult fibroblasts[6–9] has opened up a new perspective, as pluripotent stem cells can now be obtained not only from embryos but also from almost any differentiated cell. Moreover, the possibility of generating embryonic-like stem cells from adult cells let scientists speculate that generating true patient-specific therapies may become feasible. However, the fact that cells had to be reprogrammed by genetic manipulation, i.e. creating transgenic cells with retro- or lentiviruses often carrying oncogenes like c-Myc, posed serious doubts about the safety of the method. Thus, a chemical approach for the generation of stem cells from adult cells has been invoked as a safer and more convenient alternative to the genetic approach.[10] Anyhow, while the reprogramming approach was being born, several adult stem cell-based clinical trials for heart regeneration were already on-going, mainly using bone marrow stem cells.[11] Moreover, the possibility of isolating progenitor cells, also from heart specimens, led some investigators to postulate that these cells could be a better choice than bone marrow cells, as cardiac stem cells are already committed towards the heart phenotype.[12] However, results have been generally poor and quite controversial. Nonetheless, a key concept has emerged, which is that injected stem cells activate the growth and differentiation of progenitor cells that are already present in the heart, by releasing signalling factors and/or exosomes carrying them.[13] Therefore, stimulating the endogenous regenerative processes,

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possibly with a drug, could overcome the limitations and drawbacks of a cell-based approach. Moreover, understanding the mechanisms regulating cardiomyocytes turnover could be instrumental not only to repair a damaged heart, but also to increase cell resistance under adverse circumstances as ischaemia or diabetes.

In this work, these novel therapeutic approaches for heart regeneration will be critically reviewed, with particular attention to: (i) cell reprogramming using small molecules, (ii) cell differentiation stimulated by small molecules and (iii) activation of cell response by hypoxic stress. This will allow showing the original contribution to the field of the Laboratory of Stem Cells of Tissue Engineering at IRCCS Policlinico San Donato.

Chemistry for cell reprogramming

The process of cell differentiation, from embryonic stem cells (ESCs) to terminally differentiated somatic cells, has been considered for years as a one-way multistep process. In fact, even in the case of self-regeneration upon tissue damage, adult mammals can only use pre-existing progenitor cells, which are activated to proliferate and differentiate to replace the lost cells. This has been shown to be true for most tissues, including the heart, where several types of resident progenitor cells have been reported, although the results in the field are very controversial.[14] Moreover, the regenerative potential of the human heart is very limited, and it has been recently clarified that most cardiomyocytes do not regenerate during lifespan.[15] Nonetheless, there are extraordinary examples of heart regenerative capability in other animals. For instance, the zebrafish can fully regenerate its heart following amputation of up to 20% of the ventricle mainly through cardiomyocytes de-differentiation and proliferation.[16] Even the mouse has been shown to possess a higher heart regenerative potential than humans.[17] Nonetheless, if the mechanisms underlying these regenerative capabilities are elucidated, it could be envisioned to apply the insights gained to humans, perhaps by chemically activating the appropriate regenerative stimuli.[18]

Several attempts have been made over the years to unleash the same de-differentiation process in mammalians, with the ultimate goal of generating stem cells from adult cells *in vitro*. These approaches include somatic cell nuclear transfer, fusion of adult cells to ESCs, and iPSCs.[19] However, all these strategies have ethical and technical issues that still keep them far from a possible clinical application.[10] Actually, the possibility of using small synthetic molecules to activate the reprogramming process seems to have intrinsic advantages over genetic manipulation, including the possibility of adjusting the drug dosage and of suspending the treatment at any time. To date, several chemical strategies to generate iPSCs have been reported in the literature. In particular, since the WNT pathway contributes to the maintenance of pluripotency in mouse and human ESCs,[20] as well as the self-renewal of undifferentiated adult stem cells in multiple tissues,[21] several small molecules targeting this signalling pathway have been employed to increase the reprogramming efficiency of adult cells. For example, CHIR99021, an inhibitor of the GSK-3β, has been shown modulating the WNT signalling pathway, replacing c-Myc overexpression, and improving the efficiency of reprogramming.[22,23] Another important target to enhance the formation of iPSCs is TGF-β, which is directly involved in the mesenchymal–epithelial transition (MET). Mesenchymal–epithelial transition is a reversible biological process that mediates the transition from spindle-shaped mesenchymal cells to polarized epithelial cells and represents a fundamental step in the morphological changes needed for fibroblast reprogramming.[24,25] Indeed, the combined use of two small molecules, SB431542, a TGF-β receptor inhibitor, and PD0325901, an MEK inhibitor, dramatically improved (>200-folds) the efficiency of iPSC generation from human fibroblasts.[26] Within this scenario, two years before iPSCs were first reported, the synthetic purine reversine was identified from a high-throughput screen of a combinatorial library, and it was shown to be able to revert mouse myoblasts C2C12 into a more immature state, similar to that of multipotent stromal cell.[27] In fact, reversine-treated myoblasts acquired a stem cell-like phenotype, as they could be induced to differentiate into adipocytes and osteoblasts upon treatment with the appropriate differentiating media.[27] Since C2C12 are an immortal, aneuploid and tumorigenic cell line and, most importantly, they have been shown to easily transdifferentiate into osteoblasts and adipocytes, back in 2005 our research group decided to test the effects of reversine on mouse and human fibroblasts, as they are normal cells and an easily accessible cell-source for a potential therapeutic application. Moreover, these cells are easily expandable *in vitro* and maintain normal phenotype and genetic stability. Our initial study was directed to evaluate the effect of different doses of reversine on fibroblast proliferation and expression of their tissue-specific markers.[28] We also co-cultured reversine-treated fibroblasts with myogenic C2C12 cells and evaluated their ability to differentiate into skeletal muscle cells. In the same study, we also revealed the ability of reversine-treated murine fibroblasts to differentiate *in vivo*, after direct cell transplantation into cardiotoxin-injured tibialis anterior muscle of wild-type syngeneic mice. Based on these results, reversine-treated adult cells have been shown to be able to differentiate into neural cells[29] and even cardiomyocytes.[30] However, since the beginning, it was clear that understanding the mechanism of reversine-induced reprogramming was crucial to design new and better molecules. Over the past decade, our group and others have reported a possible mechanism of action of the purine. In particular, reversine has been shown to be a dual inhibitor of MEK1 and non-muscle myosin II heavy chain, inducing an alteration of the cell cycle and changes in histone acetylation status. More recently, reversine has been shown to be also an aurora kinase inhibitor, thus its potential use also as an antitumoural drug has been shown to be effective.[31] Nonetheless, in a recent report, other aurora kinase inhibitors, analogous of reversine, have been shown to induce cell reprogramming by activation of AKT-mediated phosphorylation and increase of GSK3β.[32] Therefore, further studies in this direction are currently undergoing in our laboratory.

Chemistry for cell differentiation

Finding a safe and efficient way of generating progenitor cells through the de-differentiation process is not the only field where a chemical approach could be vital. In fact, once progenitors cells are generated, it is crucial to have selective and high-yielding methods to pilot their differentiation towards the desired cell phenotype.[10] Several successful examples have already been reported, especially in the case of ESCs, and in particular, the possibility of differentiating stem/progenitor cells towards cardiomyocytes or cardiac progenitors, even with very low efficiency, has recently gathered great attention, promising new frontiers in the treatment of myocardial infarction (MI) and heart failure.[33] At this stage, small molecules may have a primary function in the development of new strategies for progenitor cell determination and differentiation. In this regard, an improvement of cardiac differentiation of pluripotent cells has been obtained by inhibition of the bone morphogenetic protein (BMP) signalling with small molecules. Bone morphogenetic proteins are involved in the regulation of several key processes underlying cardiovascular development and their temporal modulation is crucial for cardiomyogenesis.[34] For example, dorsomorphin, a molecule identified by a chemical screen in zebrafish, inhibits the BMP signalling by specifically targeting the BMP type I receptor.[35] Dorsomorphin treatment of mouse ESCs during the first day of differentiation improved (up to 30-folds) the formation of beating cardiomyocytes.[36] Another chemical inhibitor of the BMP signalling, DMH1, robustly promoted cardiomyogenesis in multiple human iPSC lines by a reproducible and faster (only 1 week) method of differentiation, supporting the importance of a chemically defined strategy to obtain large numbers of cardiomyocytes.[37]

Along this line, our group has worked on a new class of small molecules, the sulphonyl-hydrazones (SHZ), discovered by a high-throughput screening of synthetic compounds that might be a promising chemical inducer for *in vitro* and *in vivo* cardiomyogenic stimulation. Sulphonyl-hydrazone acted as an activator of the NK2 transcription factor-related (Nkx2.5), one of the earliest lineage-restricted genes to be expressed in cardiovascular progenitor cells,[38] and it was able to induce the expression of cardiac mRNAs and proteins in murine ESCs.[39] Furthermore, SHZ-pre-treated human mobilized blood mononuclear cells displayed cardioregenerative activity as xenografts in injured immunocompromised rat heart.[39]

Initially, in a collaborative study with Dr M. Sampaolesi at the Katholieke Universiteit, Leuven, Belgium, we tested SHZ effects on iPSC-derived early and late cardiomyogenesis at different concentrations, monitoring eventual effects on cell survival and apoptosis. Moreover, we examined and quantified beating foci percentages and cardiomyocyte isolation rates in order to verify whether this small chemical compound could effectively improve cardiomyogenesis of iPSCs. Results showed that 5 μM SHZ treatment was able to increase early cardiac marker expression as Nkx2.5 and GATA-binding protein 4 and late cardiac marker expression as cardiac myosin heavy chain, connexin 43, and cardiac troponin I in a dose-dependent manner, but high concentrations of the molecule induced an

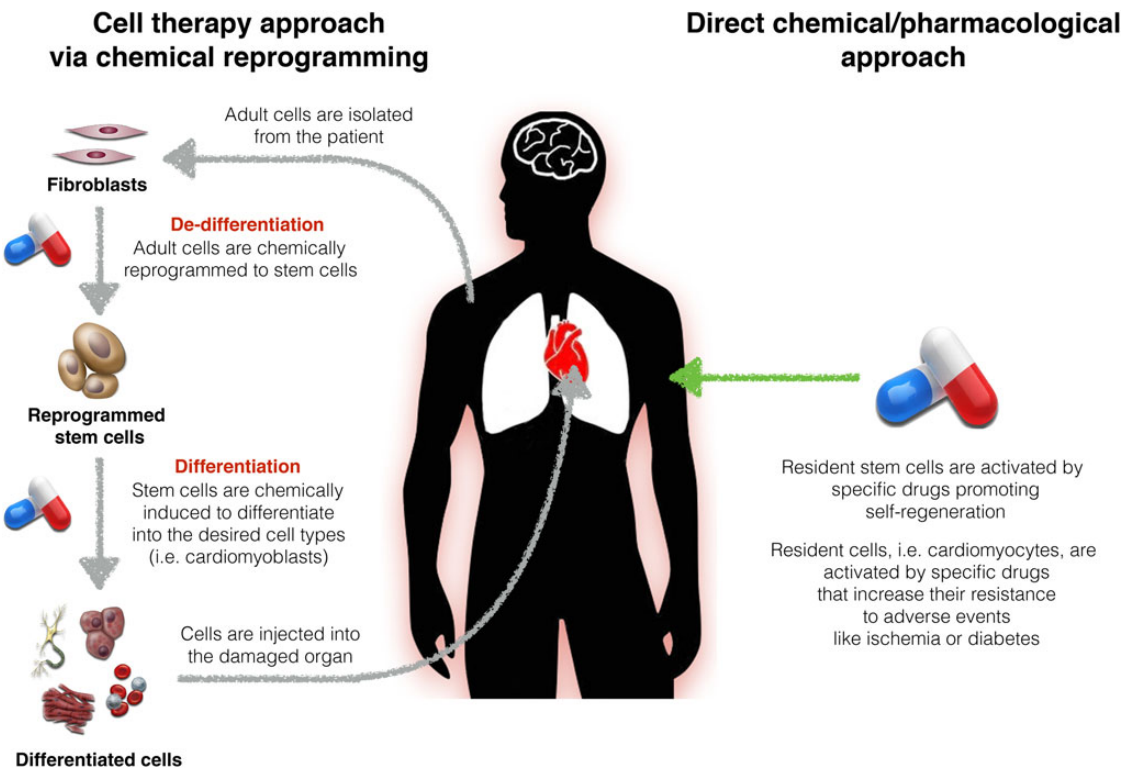

Figure 1 Schematic representation of a possible cell therapy approach via chemical reprogramming of adult cells isolated from patients and of the direct chemical/pharmacological approach for the *in situ* activation of resident cells promoting self-regeneration of tissues.

apoptosis-driven loss of viability during mid-late differentiation stages. In addition, SHZ treatment resulted in a significantly higher yield of cardiomyocytes isolated from differentiating embryoid bodies. The possible SHZ effects on beating activity were also analysed by comparing both beating cardiac foci percentages and areas of differentiation between control and SHZ-treated iPSCs. As expected, SHZ treatment of iPSCs resulted in a significant increase in beating foci rate and area extension. To exclude the hypothesis that SHZ-dependent cardiomyogenic enhancement could rely on beneficial effects on cell proliferation, we evaluated growth curves of proliferating iPSCs and isolated cardiomyocytes in the presence or absence of SHZ and we did not observe any significant variation in the proliferation rate.

Overall, our results indicated SHZ as a suitable molecule to increase *in vitro* iPSC cardiac differentiation at low concentration.[40] Undoubtedly, it would be really desirable to find small molecules that will replace or increase the efficacy of known differentiating key factors including growth factors, cytokines, and conditioning media, and these results supported the idea that a chemical approach to stem cell differentiation has started to became very effective and suitable, as many small molecules are generally not expensive, available in relatively large quantities and poorly immunogenic.[41]

Another interesting perspective for the development of new therapeutic strategies to treat heart failure includes the use of a chemical approach to *in situ* activate resident cells to regenerate the tissue, without any *in vitro* amplification steps. In fact, cardiac progenitor cells possess a very limited turnover *in vitro*, thus their direct activation in the damaged tissue may represent a valuable alternative.[10] Along this line, in our laboratory, we isolated human cardiac stromal cells from auricles obtained during cardiac surgeries of IRCCS Policlinico San Donato. These cells have been treated with SHZ before the induction of cardiac differentiation and tested for cardiac specific marker expression. Sulphonyl-hydrazone-treated cardiac stromal cells showed a 20-fold increased expression of cardiac troponin T, when compared with untreated cells (unpublished data). Even if preliminary, these results may represent an important starting point for the development of new therapeutic strategies to promote *in situ* myocardial repair/regeneration.

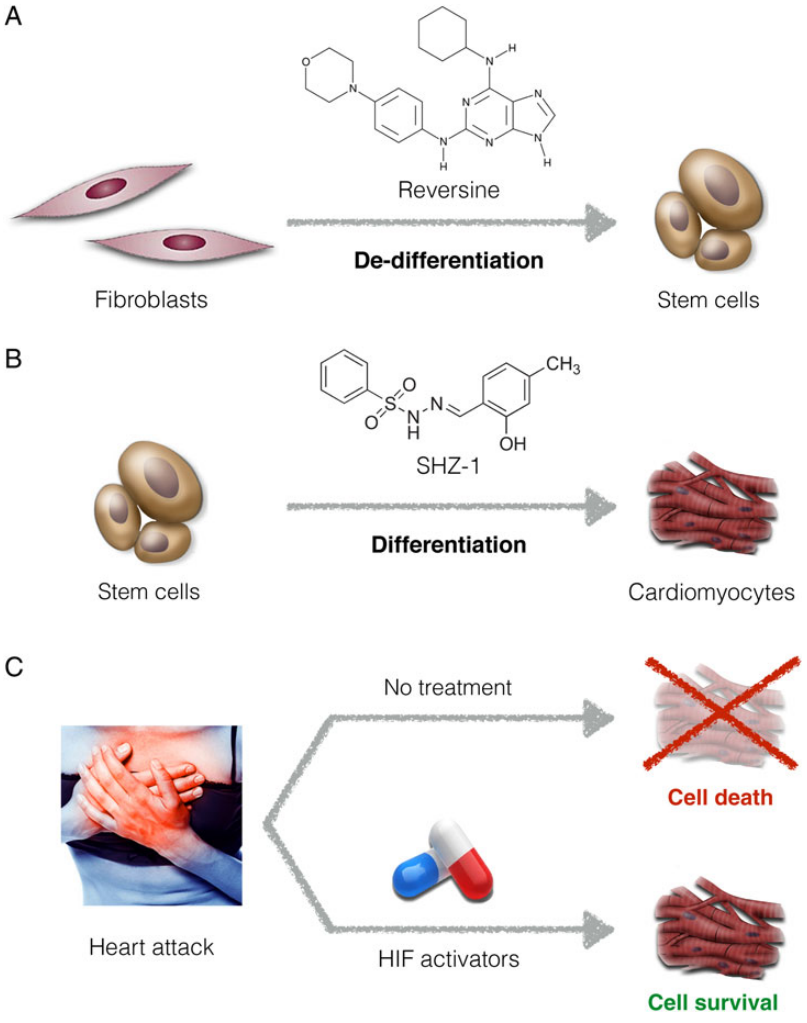

Figure 2 Examples of chemical approaches to stem cell reprogramming in cardiovascular diseases (*A*) de-differentiation approach; (*B*) differentiation approach; (*C*) cardiac diseases treatment. HIF-1α, hypoxia-inducible factor; SHZ, sulphonyl-hydrazones.

Activation of protection system

As stated in the introduction, another approach for fighting heart failure could be to increase myocyte resistance to stress. For example, ischaemic conditions lead to 'hypoxia' that causes functional impairments of cells and often structural tissue damage. A possible approach to reduce hypoxia consequences could be the activation of the hypoxia-inducible factor (HIF-1α), a transcription complex which responds to oxygen deprivation by stimulating the cell defence machinery, ultimately protecting tissues against the consequences of hypoxia.[42] Activation of HIF in mice, by genetic inhibition of its main regulator PHD2, caused preformed collateral arteries that preserved limb perfusion and prevented tissue necrosis in ischaemia.[43] It was also found that PHD2 inhibition amplifies the antioxidative response in the heart reducing the tissue damage caused by chemotherapeutic drugs.[44] Moreover, it was reported that inhibition of prolyl hydroxylases (PHDs) improved long-term ventricular function, remodelling, and vascularity after MI in a rat model.[45] While we were developing new synthetic drugs to inhibit PHD2 with Dr Mazzone, we found and reported a novel mechanism of activation of HIF-1α, not involving PHD2, and mediated by sialidase NEU3, the enzyme that releases sialic acid residues from sialoglycoconjugates (for instance from ganglioside GM3), and that we found to be triggered under hypoxia.[46] Moreover, NEU3 overexpression protects myocytes from hypoxia-induced cell death. NEU3 is a member of sialidase family, which are glycosidases that catalysed the removal of α-glycosidically liked sialic acid residues from carbohydrate group of glycoproteins and glycolipids.[47] NEU3 is known as the 'ganglioside sialidase' because it removes sialic acid preferentially from ganglioside localized on the plasma membrane, and in particular its action is targeted on ganglioside GM3.[48] In cancer, it has been demonstrated that an up-regulation of NEU3 is fundamental for the activation of EGFR pro-survival signalling pathway by reducing the content of ganglioside GM3.[49] We moved on this direction, and we focused the attention to validate the possible involvement of NEU3 in cell response to hypoxic and ischemic stress in the cardiovascular field. In our initial study, murine skeletal muscle cells, C2C12, were used to study the role of NEU3 under hypoxic conditions. Interestingly, culturing C2C12 at 1% oxygen for 72 h caused an up-regulation of NEU3. Moreover, a overexpression of NEU3 induced a marked increase of EGFR signalling cascade, thus activating its down-stream pro-survival and anti-apoptotic signalling pathways, including AKT, p70S6K, and eventually up-regulating the HIF-1α. Overall, these effects increased cell resistance to hypoxia, ultimately opposing apoptotic cell death. However, although NEU3 mechanism of action was clear, the physiological up-regulation of the enzyme under hypoxic condition was still unknown.[46] Actually, a recent study reported the binding of transcription factor SP1 and SP3 to NEU3 promoter region.[50] These factors are known to be activated under hypoxic conditions.[51] Indeed, C2C12 cells cultured under hypoxic conditions showed an up-regulation of SP1 and SP3, supporting their involvement in activating NEU3 gene transcription under low oxygen levels.[46]

Overall, these initial studies showed that NEU3 is involved in cell response to hypoxia through the down-regulation of ganglioside GM3 and the up-regulation of HIF-1α. For this reason, finding a way to mimic the action of NEU3 through the use of specific molecules could be a novel approach that may result in the development of new therapies. In this regard, our laboratory is undertaking two parallel lines of study: (i) to mimic NEU3 activity by chemically impairing GM3 biosynthesis with new sialyltransferase inhibitors and (ii) to increase HIF-1α activation by chemical inhibiting key PHDs. In particular, GM3 synthase (ST3Gal-V) belongs to the sialyltransferase family and is a unique enzyme among all sialyltransferases, since it is specifically involved in ganglioside GM3 formation.[52] In our laboratory, several GM3 synthase inhibitors have been synthesized and tested, revealing the feasibility of this approach (data submitted for publication). For the second approach, it is known that HIF-1α is tightly regulated by a family of enzymes called PHDs.[53] PHD enzymes introduce a hydroxyl group into specific prolyl residues on the HIF-α molecule. Inhibition of the PHDs has potential for the treatment of anaemia, ischaemia-related diseases, and other diseases[54,55] by enabling a range of cellular and systemic responses that enhance oxygen delivery or reduce oxygen demand. For this reason, our laboratory is screening several commercial and newly synthesized PHD inhibitors to test if they could activate cell response to hypoxia.

Overall, either NEU3 activation or PHD2 inhibition have the ultimate goal of activating cell response to stress, thus increasing their resistance to adverse conditions like ischaemia or diabetes.

In conclusion, the research activities of the Laboratory of Stem Cells for Tissue Engineering at IRCCS Policlinico San Donato are focused on the development of a chemical–pharmacological approach to heart failure. This could be achieved by activating stem cells in the heart and/or by increasing cell resistance to ischaemia (*Figures 1* and *2*). This new and original approach, which can be developed by the strict collaboration of synthetic organic chemists, biochemists, and cardiac surgeons, may lead to the generation of new drugs for the treatment of heart failure without the need of cell therapy or organ replacement.

Conflict of interest: none declared.

References

1. Senyoa SE, Lee RT, Kühnc B. Cardiac regeneration based on mechanisms of cardiomyocyte proliferation and differentiation. *Stem Cell Res* 2014; **13**:532–554.
2. Karantalis V, Hare JM. Use of mesenchymal stem cells for therapy of cardiac disease. *Circ Res* 2015;**116**:1413–1430.
3. Sadahiro T, Yamanaka S, Ieda M. Direct cardiac reprogramming: progress and challenges in basic biology and clinical applications. *Circ Res* 2015;**116**:1378–1391.
4. Izpisúa Belmonte JC, Ellis J, Hochedlinger K, Yamanaka S. Induced pluripotent stem cells and reprogramming: seeing the science through the hype. *Nat Rev Genet* 2009;**10**:878–883.

5. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;**126**:663–676.
6. Lowry WE, Richter L, Yachechko R, Pyle AD, Tchieu J, Sridharan R, Clark AT, Plath K. Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc Natl Acad Sci USA* 2008;**105**: 2883–2888.
7. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 2008;**451**:141–146.
8. 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.
9. Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE, Jaenisch R. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 2007;**448**:318–354.
10. Anastasia L, Piccoli M, Garatti A, Conforti E, Scaringi R, Bergante S, Castelvecchio S, Venerando B, Menicanti L, Tettamanti G. Cell reprogramming: a new chemical approach to stem cell biology and tissue regeneration. *Curr Pharm Biotechnol* 2011;**12**:146–150.
11. Karantalis V, DiFede DL, Gerstenblith G, Pham S, Symes J, Zambrano JP, Fishman J, Pattany P, McNiece I, Conte J, Schulman S, Wu K, Shah A, Breton E, Davis-Sproul J, Schwarz R, Feigenbaum G, Mushtaq M, Suncion VY, Lardo AC, Borrello I, Mendizabal A, Karas TZ, Byrnes J, Lowery M, Heldman AW, Hare JM. Autologous mesenchymal stem cells produce concordant improvements in regional function, tissue perfusion, and fibrotic burden when administered to patients undergoing coronary artery bypass grafting: the Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery (PROMETHEUS) trial. *Circ Res* 2014;**114**:1302–1310.
12. Chimenti I, Gaetani R, Barile L, Forte E, Ionta V, Angelini F, Frati G, Messina E, Giacomello A. Isolation and expansion of adult cardiac stem/progenitor cells in the form of cardiospheres from human cardiac biopsies and murine hearts. *Methods Mol Biol* 2012;**879**: 327–338.
13. Ailawadi S, Wang X, Gu H, Fan GC. Pathologic function and therapeutic potential of exosomes in cardiovascular disease. *Biochim Biophys Acta* 2015;**1852**:1–11.
14. Van Berlo JH, Kanisicak O, Maillet M, Vagnozzi RJ, Karch J, Lin SC, Middleton RC, Marbán E, Molkentin JD. c-kit+ cells minimally contribute cardiomyocytes to the heart. *Nature* 2014;**509**:337–341.
15. Bergmann O, Zdunek S, Felker A, Salehpour M, Alkass K, Bernard S, Sjostrom SL, Szewczykowska M, Jackowska T, Dos Remedios C, Malm T, Andrä M, Jashari R, Nyengaard JR, Possnert G, Jovinge S, Druid H, Frisén J. Dynamics of cell generation and turnover in the human heart. *Cell* 2015;**161**:1566–1575.
16. Jopling C, Sleep E, Raya M, Martí M, Raya A, Izpisúa Belmonte JC. Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. *Nature* 2010;**464**:606–609.
17. Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, Sadek HA. Transient regenerative potential of the neonatal mouse heart. *Science* 2011;**331**:1078–1080.
18. Kikuchi K. Dedifferentiation, transdifferentiation, and proliferation: mechanisms underlying cardiac muscle regeneration in zebrafish. *Curr Pathobiol Rep* 2015;**3**:81–88.
19. Hu K. All roads lead to induced pluripotent stem cells: the technologies of iPSC generation. *Stem Cells Dev* 2014;**23**:1285–1300.
20. Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat Med* 2004;**10**:55–63.
21. Niwa H. Wnt: what's needed to maintain pluripotency? *Nat Cell Biol* 2011;**13**:1024–1026.
22. Lin T, Wu S. Reprogramming with small molecules instead of exogenous transcription factors. *Stem Cells Int* 2015;**2015**:794632.
23. Marson A *et al. Wnt signaling promotes reprogramming of somatic cells to pluripotency. Cell Stem Cell* 2008;**3**:132–135.
24. Li R, Liang J, Ni S, Zhou T, Qing X, Li H, He W, Chen J, Li F, Zhuang Q, Qin B, Xu J, Li W, Yang J, Gan Y, Qin D, Feng S, Song H, Yang D, Zhang B, Zeng L, Lai L, Esteban MA, Pei D. A mesenchymal-to-epithelial transition initiates and is required for the nuclear reprogramming of mouse fibroblasts. *Cell Stem Cell* 2010;**7**:51–63.
25. Samavarchi-Tehrani P, Golipour A, David L, Sung HK, Beyer TA, Datti A, Woltjen K, Nagy A, Wrana JL. Functional genomics reveals a BMP-driven mesenchymal-to-epithelial transition in the initiation of somatic cell reprogramming. *Cell Stem Cell* 2010;**7**:64–77.
26. Lin T, Ambasudhan R, Yuan X, Li W, Hilcove S, Abujarour R, Lin X, Hahm HS, Hao E, Hayek A, Ding S. A chemical platform for improved induction of human iPSCs. *Nat Methods* 2009;**6**:805–808.
27. Chen S, Zhang Q, Wu X, Schultz PG, Ding S. Dedifferentiation of lineage-committed cells by a small molecule. *J Am Chem Soc* 2004;**126**: 410–411.
28. Anastasia L, Sampaolesi M, Papini N, Oleari D, Lamorte G, Tringali C, Monti E, Galli D, Tettamanti G, Cossu G, Venerando B. Reversine-treated fibroblasts acquire myogenic competence in vitro and in regenerating skeletal muscle. *Cell Death Differ* 2006;**13**:2042–2051.
29. Lee EK, Bae GU, You JS, Lee JC, Jeon YJ, Park JW, Park JH, Ahn SH, Kim YK, Choi WS, Kang JS, Han G, Han JW. Reversine increases the plasticity of lineage-committed cells toward neuroectodermal lineage. *J Biol Chem* 2009;**284**:2891–2901.
30. Pikir BS, Susilowati H, Hendrianto E, Abdulrantam F. Reversin increase the plasticity of bone marrow-derived mesenchymal stem cell for generation of cardiomyocyte in vitro. *Acta Med Indones* 2012;**44**:23–27.
31. D'Alise AM, Amabile G, Iovino M, Di Giorgio FP, Bartiromo M, Sessa F, Villa F, Musacchio A, Cortese R. Reversine, a novel Aurora kinases inhibitor, inhibits colony formation of human acute myeloid leukemia cells. *Mol Cancer Ther* 2008;**7**:1140–1149.
32. Li Z, Rana T. A kinase inhibitor screen identifies small-molecule enhancers of reprogramming and iPS cell generation. *Nat Commun* 2012;**3**: 1085.
33. Chen A, Ting S, Seow J, Reuveny S, Oh S. Considerations in designing systems for large scale production of human cardiomyocytes from pluripotent stem cells. *Stem Cell Res Ther* 2014;**21**:12.
34. van Wijk B, Moorman AF, van den Hoff MJ. Role of bone morphogenetic proteins in cardiac differentiation. *Cardiovasc Res* 2007;**74**:244–255.
35. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest* 2001;**108**:1167–1174.
36. Hao J, Daleo MA, Murphy CK, Yu PB, Ho JN, Hu J, Peterson RT, Hatzopoulos AK, Hong CC. Dorsomorphin, a selective small molecule inhibitor of BMP signaling, promotes cardiomyogenesis in embryonic stem cells. *PLoS ONE* 2008;**3**:e2904.
37. Aguilar JS, Begun AN, Alvarez J, Zhang XB, Hong Y, Hao J. Directed cardiomyogenesis of human pluripotent stem cells by modulating Wnt/beta-catenin and BMP signalling with small molecules. *Biochem J* 2015;**469**:235–241.
38. Garry DJ, Olson E. A common progenitor at the heart of development. *Cell* 2006;**127**:1101–1104.
39. Sadek H, Hannack B, Choe E, Wang J, Latif S, Garry MG, Garry DJ, Longgood J, Frantz DE, Olson EN, Hsieh J, Schneider JW. Cardiogenic small molecules that enhance myocardial repair by stem cells. *Proc Natl Acad Sci* 2008;**105**:6063–6068.
40. Quattrocelli M, Palazzolo G, Agnolin I, Martino S, Bouché M, Anastasia L, Sampaolesi M. Synthetic sulfonyl-hydrazone-1 positively regulates cardiomyogenic microRNA expression and cardiomyocyte differentiation of induced pluripotent stem cells. *J Cell Biochem* 2011;**112**:2006–2014.
41. Anastasia L, Pelissero G, Venerando B, Tettamanti G. Cell reprogramming: expectations and challenges for chemistry in stem cell biology and regenerative medicine. *Cell Death Differ* 2010;**17**:1230–1237.
42. Miyata T, Takizawa S, van Ypersele de Strihou C. Intracellular sensors for oxygen and oxidative stress: novel therapeutic targets. *Am J Physiol Cell Physiol* 2011;**300**:c226–c231.
43. Takeda Y, Costa S, Delamarre E, Roncal C, Leite de Oliveira R, Squadrito ML, Finisguerra V, Deschoemaeker S, Bruyère F, Wenes M, Hamm A, Serneels J, Magat J, Bhattacharyya T, Anisimov A, Jordan BF, Alitalo K, Maxwell P, Gallez B, Zhuang ZW, Saito Y, Simons M, De Palma M, Mazzone M. Macrophage skewing by Phd2 haplodeficiency prevents ischaemia by inducing arteriogenesis. *Nature* 2011; **479**:122–126.
44. Leite de Oliveira R, Deschoemaeker S, Henze AT, Debackere K, Finisguerra V, Takeda Y, Roncal C, Dettori D, Tack E, Jönsson Y, Veschini L, Peeters A, Anisimov A, Hofmann M, Alitalo K, Baes M, D'hooge J, Carmeliet P, Mazzone M. Gene-targeting of Phd2 improves tumor response to chemotherapy and prevents side-toxicity. *Cancer Cell* 2012;**22**:263–277.
45. Bao W, Qin P, Needle S, Erickson-Miller CL, Duffy KJ, Ariazi JL, Zhao S, Olzinski AR, Behm DJ, Pipes GC, Jucker BM, Hu E, Lepore JJ,

Willette RN. Chronic inhibition of hypoxia-inducible factor prolyl 4-hydroxylase improves ventricular performance, remodeling, and vascularity after myocardial infarction in the rat. *J Cardiovasc Pharmacol* 2010;**56**:147–155.
46. Scaringi R, Piccoli M, Papini N, Cirillo F, Conforti E, Bergante S, Tringali C, Garatti A, Gelfi C, Venrando B, Menicanti L, Tettamenti G, Anastasia L. NEU3 sialidase is activated under hypoxia and protects skeletal muscle from apoptosis through the activation of the epidermal growth factor receptor signaling pathway and the hypoxia-inducible factor (HIF-1α). *J Biol Chem* 2013;**288**:3153–3162.
47. Miyagi T, Wada T, Iwamatsu A, Hata K, Yoshikawa Y, Tokuyama S, Sawada M. Molecular cloning and characterization of a plasma membrane-associated sialidase specific for ganglioside. *J Biol Chem* 1999;**274**:5004–5011.
48. Miyagi T, Yamaguchi K. Mammalian sialidases: physiological and pathological roles in cellular functions. *Glycobiology* 2012;**22**: 880–896.
49. Miyagi T. Aberrant expression of sialidase amd camcer progression. *Proc Jnp Acad Ser B Phys Biol Sci* 2008;**84**:407–418.
50. Yamaguchi K, Koseki K, Shiozaki M, Shimada Y, Wada T, Miyagi T. Regulation of plasma membrane-associated sialidase NEU3 gene by Sp1/Sp3 transcription factors. *Biochem J* 2010;**430**:107–117.
51. Cummins EP, Taylor CT. Hypoxia-responsive transcription factors. *Pflugers Arch* 2005;**430**:363–371.
52. Kono M, Takashima S, Liu H, Inoue M, Kojima N, Lee YC, Hamamoto T, Tsuji S. Molecular cloning and functional expression of a fifth-type alpha 2,3-sialyltransferase (mST3Gal V: GM3 synthase). *Biochem Biophys Res Commun* 1998;**253**:170–175.
53. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, von Kriegsheim A, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. *Science* 2001;**292**:468–472.
54. Hewitson KS, Schofield CJ. The HIF pathway as a therapeutic target. *Drug Discov Today* 2004;**9**:704–711.
55. Nagel S, Talbot N, Mecinović J, Smith TG, Buchan AM, Schofield CJ. Therapeutic manipulation of the HIF hydroxylases. *Antioxid Redox Signal* 2010;**12**:481–501.