Cell Regeneration 7 (2018) 1–6

Contents lists available at ScienceDirect

Cell Regeneration

journal homepage: http://www.keaipublishing.com/en/journals/cell-regeneration/

Review

# Cardiac progenitor reprogramming for heart regeneration

Behnam Ebrahimi*

*Yazd Cardiovascular Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran*

ARTICLE INFO

*Article history:*
Received 13 September 2017
Received in revised form
21 December 2017
Accepted 2 January 2018
Available online 12 February 2018

*Keywords:*
Cellular reprogramming
Transdifferentiation
Myocardial infarction
Heart regeneration

ABSTRACT

Myocardial infarction leads to the loss of a huge number of cardiomyocytes and the reparatory response to this phenomenon is scar tissue formation, which impairs heart function. Direct reprogramming technology offers an alternative strategy for the generation of functional cardiomyocytes not only *in vitro*, but also *in vivo* in the site of injury. Results have demonstrated cardiac tissue regeneration and improvement in heart function after myocardial infarction following local injection of vectors encoding reprogramming transcription factors or miRNAs. This shows the great potential of cardiac reprogramming technology for heart regeneration. However, in addition to cardiomyocytes, other cell types, including endothelial cells and smooth muscle cells are also required to be generated in the damaged area in order to achieve complete cardiac tissue regeneration. To this aim induced proliferative/expandable cardiovascular progenitor cells (iCPCs) appear to be an appropriate cell source, which is capable of differentiation into three cardiovascular lineages both *in vitro* and *in vivo*. In this regard, this study goes over *in vitro* and *in vivo* cardiac reprogramming technology and specifically deals with cardiac progenitor reprogramming and its potential for heart regeneration.


## 1. Introduction

Cardiac diseases are the major cause of morbidity and take the lives of millions of people worldwide.[1] Due to the inadequacy of heart for regeneration, cardiac injury results in excessive fibrosis and consequently adverse remodeling and impairment of heart function.[2] Regarding the large number of cardiac fibroblasts[3,4] and feasibility of *in vitro* reprogramming of fibroblasts into induced cardiomyocytes (iCMs), *in vivo* conversion of resident cardiac fibroblasts into iCMs has been found a promising strategy for induced regeneration.[5] Multiple effective efforts have been accomplished and confirmed the potential of direct reprogramming of cardiac fibroblasts of the infarcted area into iCMs for heart regeneration and improvement in heart function.[6] These findings illustrate the importance of direct cardiac reprogramming as a new technology and its promise for future regenerative cardiac therapies in human.

Nevertheless, it should be admitted that mature cardiomyocytes cannot completely replenish injured area while regeneration needs a variety of cell types. In this respect, cardiac-fate restricted progenitor cells capable of differentiation to three cardiovascular lineages appear to be required to be induced *in situ*. Accordingly, *in situ* production of induced cardiac progenitor cells (iCPCs) may achieve more success than the cardiomyocyte reprogramming. This is due to their potential for differentiation into three major cardiac cell types, which can provide the injured heart with both contractile cells and blood vessels.[7,8] Thus, lineage-restricted induced progenitors seem to have superiority over the terminally differentiated iCMs for reconstruction of a diseased or damaged tissue.

The current study reviews what is known about *in vitro and in vivo* direct cardiac reprogramming and specifically discusses direct reprogramming into cardiac progenitor cells and its superiority over terminally specialized cardiomyocytes for heart regeneration.

*Abbreviations:* iCMs, induced cardiomyocytes; iCPCs, induced cardiac progenitor cells; iCSs, induced cardiospheres; TF, transcription factor; iPSC, induced pluripotent stem cell; GMT, Gata4, Mef2c, and Tbx5; CASD, cell-activation and signaling-directed; ECs, endothelial cells; SMCs, smooth muscle cells; p38 MAPK, p38 mitogen-activated protein kinase pathway; PI3K/AKT, phosphoinositol 3-kinase pathway; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor.

* Yazd Cardiovascular Research Center, Afshar hospital, Jomhouri boulevard, Yazd, Iran. Fax: +983535231421.
*E-mail addresses:* bhnmebrahimi@yahoo.com, ebrahimi.b@ssu.ac.ir.
Peer review under responsibility of Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences.

https://doi.org/10.1016/j.cr.2018.01.001

## 2. Direct reprogramming into cardiomyocytes

Ectopic expression of lineage-instructive transcription factors was first shown capable of direct cellular reprogramming by Weintraub laboratory in 1986. Weintraub and colleagues revealed that mouse fibroblasts can be converted into stable myoblasts by transcription factor (TF) MyoD.[9,10] Then, in a pioneering attempt for *in vivo* direct reprogramming, in 1996, Murry and colleagues indicated that injection of *MyoD* adenovirus into rat cardiac granulation tissue can induce expression of myogenin and embryonic MHC (embryonic myofiber phenotype) in a limited number of cells.[11]

In 2006, direct reprogramming re-attracted attentions by the advent of induced pluripotent stem cell (iPSC) technology.[12] To date, various lineage-specific TFs have been identified and a variety of cell types have been produced using this approach.[5,13] Notably, transcription factors are chosen according to their role during heart development and during a process of trial and elimination a minimum set of factors are selected to induce cardiac fate in different types of somatic cells (e.g. embryonic and adult skin and cardiac fibroblasts).

In the field of cardiac reprogramming, a number of efforts have been accomplished for the production of iCMs from fibroblasts. In 2010, Ieda et al[14] found that a combination of three cardiac TFs, including Gata4, Mef2c, and Tbx5 (GMT) can transdifferentiate mouse postnatal cardiac and dermal fibroblasts into functional iCMs *in vitro*. Afterwards, Song et al supplemented GMT with Hand2 and exhibited that GHMT reprograms adult murine fibroblasts into contracting iCMs *in vitro* with more efficiency than GMT.[15] Meanwhile, Jayawardena et al displayed that miRNAs 1, 133, 208, and 499a, in a single transient transfection, can reprogram mouse cardiac fibroblasts to functional iCMs and that JAK inhibitor I can improve efficiency of the process up to 10 fold.[16] Exploring the effect of miRNAs on the TF mediated reprogramming, Muraoka et al reported that addition of miR-133a to GMT (GMT/miR-133a) enhances cardiac reprogramming of mouse fibroblasts in terms of kinetics and efficiency by suppressing fibroblast program, which is an important barrier of cardiac reprogramming.[17] This method yielded 7-fold more beating iCMs in comparison with GMT alone.

Interestingly, Ding and colleagues developed a new approach named cell-activation and signaling-directed (CASD) lineage conversion[18,19] to convert mouse fibroblasts into cardiomyocytes. To elaborate, they induced a plastic state in mouse fibroblasts by transient expression of pluripotency factors (Oct4, Sox2, Klf4, and c-Myc; OSKM) and then lineage-specific signals (small molecule Janus kinase (JAK) inhibitor) were employed to establish cardiac fate in OSKM induced cells. In 2014, they redressed their CASD method and reprogrammed mouse adult and embryonic fibroblasts into spontaneously contracting iCMs using Oct4 alone and a small molecule cocktail consists of CHIR99021 (Wnt activator), SB431542 (TGF-β inhibitor), Parnate, and Forskolin (SCPF).[20] This data validated the viability of application of small molecules in the precise establishment of cardiac fate.

To improve the efficiency and quality of cardiac reprogramming process multiple endeavors have been completed by experimenting different TFs and environmental cues. In this respect, Protze et al[21] found that up-regulation of a wider range of cardiac genes can be achieved by overexpression of Tbx5, Mef2c, and Myocd rather than GMT. Although produced iCMs didn't beat, they expressed cardiac contractile proteins and generated cardiac-like sodium and potassium currents and action potentials. Then, Addis and colleagues demonstrated that addition of Hand2 and Nkx2.5 to original GMT (HNGMT) converts mouse fibroblasts into cardiomyocytes 50 times more efficient than GMT alone.[22]

Accelerating direct reprogramming, Hirai et al fused GMT and Hand2 (GHMT) with MyoD transactivation domain and showed that chimeric Mef2c with the wild-types of Gata4, Tbx5 and Hand2 can produce not only larger beating clusters of iCMs, but also quicker and with more efficiency (15-fold) than the four wild-type genes.[23] Identification and inhibition of reprogramming barriers will increase the efficiency of the process[24,6] and close this technology to the clinical setting. Detecting TGF-β signaling pathway as a barrier of cardiac reprogramming, Ifkovits et al,[25] meanwhile, displayed that chemical inhibition of TGF-β by SB432542 can improve five times the efficiency of HNGMT-mediated reprogramming of embryonic and adult fibroblasts. In this respect, Song and colleagues found that inhibition of pro-fibrotic signaling using small molecule inhibitors of RhoA-ROCK or TGF-β signaling considerably enhances the efficiency of GHMT plus miR-1 and miR-133 (GHMT2m) up to 60% in the conversion of mouse embryonic and adult fibroblast into beating cardiomyocytes.[26] This finding provides a strategy for highly efficient and rapid reprogramming to beating cardiomyocyte by chemical suppression of pro-fibrotic signaling.[26]

Obviating the need for further genetic manipulations and also undefined effects of serum, which can reduce reprogramming efficiency, Yamakawa et al developed a serum-free medium containing a combination of FGF2, FGF10, and VEGF, named FFV that can greatly increase the quality of the mouse GMT reprogramming.[27] Moreover, FFV in the presence of IWP4, an inhibitor of Wnt signaling, increased the efficiency by approximately 100-fold compared to the conventional FBS condition. Mechanistically, acting through the p38MAPK and PI3K/AKT pathways, FFV enhanced efficiency by rising conversion of partially reprogrammed iCMs into mature functional iCMs.

In a recent study, Olson and colleagues in a study found that addition of Akt 1 to GHMT factors (AGHMT) enhances quantity and quality of transdifferentiation.[28] They reported that Notch signaling is a roadblock to cardiac reprogramming and that its inhibition improves the process. Interestingly, in a cooperative manner, Notch inhibition and Akt1 activation boosted the efficiency of the method up to 70%, while 45% of the generated cardiomyocytes showed spontaneous beating.[29]

Toward an integration-free reprogramming approach, Fu et al reprogrammed mouse fibroblasts into spontaneously contracting iCMs using only chemical cocktails in a two-stage reprogramming strategy.[30] Avoiding the use of viral vectors, this can be considered as a safer method for iCM production for potential clinical applications.

Collectively, these data illustrate the applicability of direct cardiac reprogramming by different methods, which suggests ideas for drug testing, disease modeling, cell therapy and *in vivo* reprogramming.

## 3. *In vivo* cardiac reprogramming

Promising results of *in vitro* cardiac reprogramming have led scientists to investigate the applicability of direct cardiac reprogramming *in vivo*. In 2012, four independent laboratories reported encouraging results of *in vivo* cardiac transdifferentiation in mouse myocardial infarction models.[15,16,6,31–33] In this regard, Qian et al reported that injection of retroviral vectors encoding GMT into peri-infarct areas of mouse heart can reprogram resident cardiac fibroblasts into functional iCMs. The newly generated cells were electrically mature and improved heart function 3 months after MI. Similarly, Song et al revealed that non-cardiomyocyte cells of an infarcted myocardium can be converted into functional cardiomyocytes by GMT plus *Hand2* (GHMT) more efficient than GMT.[15] GHMT also diminished scar size and improved heart function 12 weeks after MI. Confirming these studies, Inagawa et al indicated successful reprogramming of resident non-myocytes into

iCMs using a polycistronic vector encoding GMT.[31] In detail, regarding the notion that a single-promoter polycistronic vector can achieve higher efficiencies than separate viral vectors[31,34,35] and to avoid heterogeneous and imperfect delivery of genes, single polycistronic retroviral vectors expressing GMT were used to induce cardiac reprogramming *in vivo* in mouse[31] and rat[34] hearts following MI.

Then, it was shown that *in vivo* cardiac reprogramming can be achieved by lentiviral vectors encoding miR-1, −133, −208, and −499 (miR combo).[16] Jayawardena et al proved that miRNA reprogrammed cells are functional mature cardiomyocytes and that this method improved heart contractile function gradually during 6 months.[36] This indicates that miRNA-mediated reprogramming can be a therapeutic approach to induce cardiac regeneration after injury.

Improving the efficiency of reprogramming, Qian and colleagues found that stoichiometric expression of G, M, and T influences the efficiency of reprogramming and the quality of iCMs.[37] They indicated that a polycistronic vector expressing a moderately high level of M and low level of G and T (MGT) can increase reprogramming efficiency and also heart function more than the combined separate G/M/T viruses.[35] Thus, one factor that can affect reprogramming is a homogeneous and stoichiometric expression of reprogramming factors.

Recently, Srivastava and colleagues improved the process by chemical inhibition of cardiac reprogramming barriers. After intramyocardial injection of GMT into infarcted hearts, they intraperitoneally injected small-molecules SB431542 (TGF-β inhibitor) and XAV939 (WNT inhibitor) every day for 2 weeks. This treatment considerably improved the efficiency of *in vivo* cardiac reprogramming, remuscularization and heart function compared with GMT alone. This finding reveals the importance of the application of small molecules as inhibitors of barriers and enhancers of induced regeneration. However, more research is required toward a chemical-only *in vivo* cardiac reprogramming and regeneration.

## 4. Cardiac progenitor reprogramming

Production of cardiomyocytes through direct reprogramming is a viable strategy for heart regeneration and to date multiple studies have reported successful reprogramming of fibroblasts into iCMs.[14,16,22] However, terminally differentiated cardiomyocytes are not the only cell type which is required for heart regeneration and they cannot repopulate and robustly replenish damaged area. Suggestively, a cardiac-fate restricted progenitor cell type capable of generating three cardiovascular lineages is desired to be induced. To enlighten, in addition to the generation of contractile cardiac cells, production of a functional cardiac tissue needs other cell types, including endothelial cells and smooth muscle cells that are building blocks of blood vessels. To this aim, induced proliferative/expandable cardiovascular progenitor cells (iCPCs) appear to be an appropriate cell source.[7,8] Recently, several studies reported reprogramming of fibroblasts into iCPCs and also their potential for heart regeneration by differentiation into three cardiovascular lineages when transplanted into the infarcted area (Table 1, Fig. 1).

In 2012, Islas et al showed that lentivirus-mediated forced expression of transcription factor ETS2 for one week converts normal human dermal fibroblasts into FLK1$^+$ and PECAM1$^+$ highly replicative small rounded cells, which is of the characteristics of endothelial and cardiac progenitors.[38] Furthermore, overexpression of MESP1 activated the appearance of FLK1 and PECAM1 without significant change in the shape of fibroblasts. This finding shows that ETS2 or MESP1 could induce cardiovascular gene expression in human fibroblasts. Indeed, these FLK1$^+$, PECAM1$^+$ and Nkx2.5$^+$ cells are cardiovascular progenitors with potential for differentiation into cardiomyocytes (CMs), endothelial cells (ECs), and smooth muscle cells (SMCs) and are not specifically committed to terminal cardiogenesis.[38–40] Then, to induce cardiomyocyte fate in human fibroblasts, they found that co-expression of ETS2 and MESP1 for 4 days followed by 2 days administration of Activin A and BMP2 can significantly increase expression of mesoderm signaling factors and cardiac-specified genes in human fibroblasts and reprogram them directly into cardiomyocyte progenitors or cardiac immature myocytes. In addition, avoiding concerns in respect with detrimental rearrangements of the host chromosomes during viral integration, Islas et al fused ETS2 and MESP1 to a cell-penetrating peptide, transactivator of transcription (TAT), and treated the cells with TAT-ETS2 and TAT-MESP1 proteins for four days and then with Activin A and BMP2 for two days. Colonies of cellular aggregates appeared within 8 days and about 8 percent of the cells were positive for FLK/PECAM1 cell surface markers representing cardiovascular progenitor cells.[38]

In 2015, Li et al. indicated that protein-transduction of GHMT (Gata4, Hand2, Mef2c, and Tbx5) in combination with growth factors, BMP4, activin A, and bFGF rapidly and efficiently reprograms human dermal fibroblasts (HDFs) into iCPCs with tri-lineage cardiovascular differentiation potential. Moreover, these human iCPCs demonstrated functional properties similar to native cardiac progenitors and improved cardiac function when transplanted into the infarcted rat hearts.[41]

Extending efforts to reveal applicability and regenerative capacity of cardiac progenitor reprogramming, in 2016, two different groups independently reported production of iCPCs using two different reprogramming approaches.[7,8] In a study, Lalit et al showed that five cardiac factors (MTGNB; *Mesp1*, *Tbx5*, *Gata4*, *Nkx2.5*, and *Baf60c*) in conjunction with Wnt and JAK/STAT signaling pathways are sufficient to reprogram cardiac fibroblasts of the adult mouse heart into stably proliferative and tripotent

**Table 1**
Selected reports of cardiac progenitor reprogramming.

| Starting cells | Delivery system | Reprogramming factors | Product | Ref |
|---|---|---|---|---|
| Human normal dermal fibroblasts | Lentivirus or TAT-ETS2 and TAT-MESP1 Proteins | ETS2 and MESP1 plus Activin A and BMP2 | iCPCs | 38 |
| Human Adult Dermal Fibroblasts | Nonviral, QQ-reagent based protein delivery system | GHMT using QQ-reagent together with three cytokines (BMP4, activin A and bFGF) | iCPCs | 41 |
| Secondary mouse embryonic fibroblasts | Doxycycline inducible Oct4, Sox2, Klf4 and Myc (OSKM) | Step 1. Doxycycline + JI1<br>Step 2. CHIR99021+ JI1<br>Step 3. BACS (BMP4, Activin A, CHIR99021, and SU5402) and JI1 | iCPCs | 8 |
| Adult mouse cardiac fibroblasts<br>Adult lung fibroblasts<br>Adult tail tip fibroblasts | Lentivirus | *Mesp1*, *Tbx5*, *Gata4*, *Nkx2.5*, and *Baf60c* plus 6-bromoindirubin-30-oxime (BIO, a canonical Wnt activator) and LIF (a JAK/STAT activator | iCPCs | 7 |
| Embryonic and adult mouse fibroblasts.<br>Human skin fibroblasts | Retrovirus | Step 1: Oct4, Sox2 and Klf4 overexpression.<br>Step 2: Gsk3β inhibitor BIO and Oncostatin M | iCSs | 43 |

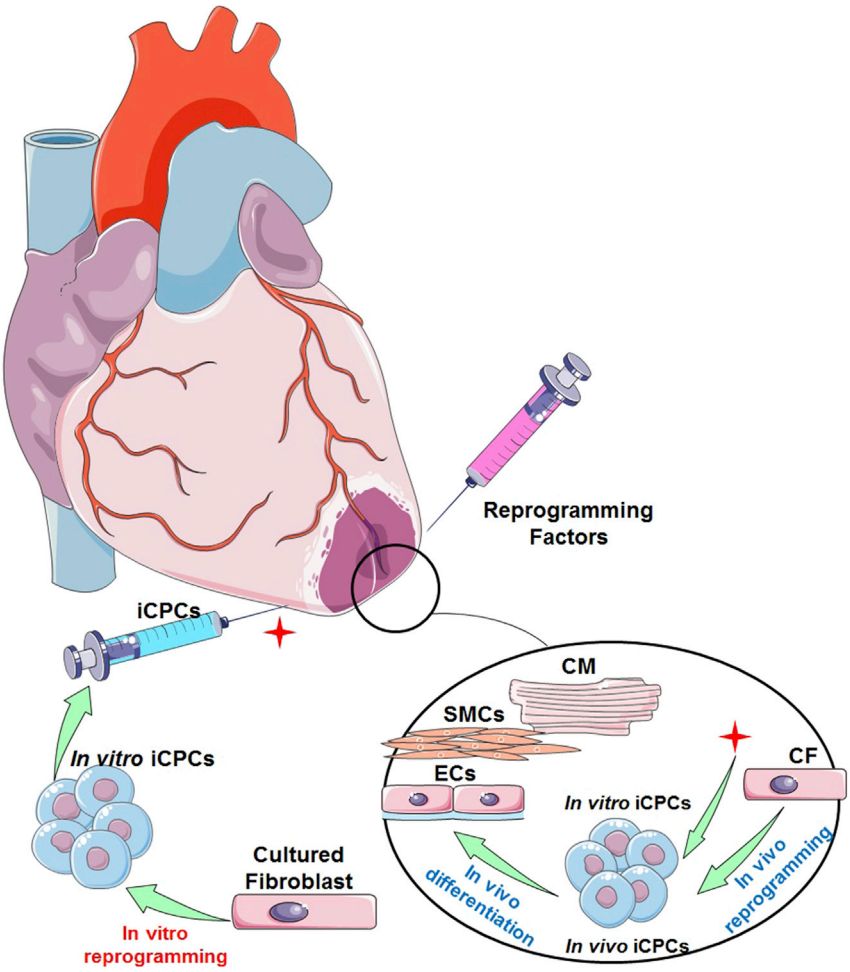


**Fig. 1.** The diagram represents cardiac progenitor reprogramming as a new approach for heart regeneration. Patient's somatic fibroblasts can be used as the starting cells for the generation of iCPCs *in vitro*, which can be injected into the heart in the next step (left part). The right section shows *in vivo* differentiation of iCPCs and also *in vivo* cardiac progenitor reprogramming as a possible strategy for induction of regeneration within the adult heart. CM: cardiomyocyte; SMCs: smooth muscle cells; ECs: endothelial cells; CF: cardiac fibroblasts; iCPCs: induced cardiac progenitor cells.

Flk1$^+$, PDGFR$\alpha^+$, and Isl1$^+$ iCPCs. These iCPCs were cardiac mesoderm-restricted progenitors with potential for differentiation to CMs, ECs, and SMCs *in vitro* and *in vivo*.[7] Interestingly, when injected into the cardiac crescent of developing mouse embryos, iCPCs integrated into the heart tube and differentiated into CMs. Furthermore, after injection into post-MI mouse hearts, they improved survival rate and differentiated into functional cardiomyocytes. In addition, iCPCs contributed to the vasculature within the scar tissue by differentiation into SMCs, and ECs.[7]

The other study by Zhang et al. used CASD lineage conversion method to convert mouse fibroblasts into iCPCs using pluripotency factors and a chemically defined condition.[8,19] These iCPCs were Flk1$^+$, PDGFR$\alpha^+$, Isl1$^+$, and Nkx2.5$^+$ expandable committed cardiovascular precursor cells with restricted cardiovascular potentials to differentiate into three cardiovascular cell types. These progenitor cells successfully differentiated into functional CMs, ECs, and SMCs *in vitro* and *in vivo*. Interestingly, similar to the iCPCs generated by Lalit et al, after their transplantation into the infarcted myocardium, iCPCs spontaneously differentiated into CMs, ECs, and SMCs and improved cardiac function. Although at a low frequency, transplanted cells also contributed to the formation of blood vessels.[8]

Toward the aim of heart regeneration, one approach is to inject cultured and expanded iCPCs directly into the damaged area, which seems to be demanding regarding fibroblast isolation/culture, reprogramming process, iCPCs expansion and then their injection. In addition, suggestively, iCPCs could be induced *in vivo* by injection of reprogramming factors into the myocardium similar to *in vivo* iCMs. However, this is an interesting topic worthy of investigation. In this regard, to estimate, among these approaches, the methods of Lalit et al and Islas et al seem to be more applicable to be adopted *in vivo* than the multi-step CASD approach. Nevertheless, the use of viral vectors limits clinical application of this approach. On the other hand, translation of the CASD transdifferentiation into *in vivo* models needs to resolve several challenges, including optimization of duration and concentrations of chemicals/growth factors, delivery routes, and its stepwise procedure. However, in respect to the advantages of the CASD, which utilizes chemicals, it should be noted that chemical reprogramming is more ideal for regenerative medicine purposes than genetic manipulation by viruses. Together, each method has its own specific merits and demerits for its direct *in vivo* application.

Cardiospheres are a kind of cardiac progenitors that are potentially safe and effective for regeneration of the infarcted hearts.[42–45] Xu et al using the CASD transdifferentiation method reprogrammed mouse and human fibroblasts into induced cardiospheres (iCSs).[43] In detail, in the first step, mouse embryonic and adult skin fibroblasts were infected overnight with retroviral vectors encoding Oct4, Sox2 and Klf4. Then, they replaced medium with Knockout Serum Replacement-based medium for 18 days.

Starting from day 16 of reprogramming, the cells experienced an increase in the expression of cardiac progenitor markers Isl1, Nkx2.5 and Mesp1 as well as cardiac marker Gata4. In the signaling-directed step, from day 18 of reprogramming, the cells were treated with GSK3β inhibitor-BIO and Oncostatin M (OSM) for 2 days. Combination of these two factors considerably augmented the mRNA level of Mesp1, Isl1, and Nkx2.5 by increasing the protein level of β-Catenin and sphere formation appeared after BIO and OSM administration.[43]

To induce cardiomyocyte differentiation in iCSs, they were treated with differentiation medium (DMEM/High Glucose, 15% FBS, GlutaMAX, nonessential amino acids, 2-mercaptoethanol, Ascorbic acid) for 15 days and beating colonies appeared at day 12 after differentiation. This approach was also successful in the conversion of human skin fibroblast into iCSs.[43] Interestingly, transplantation of iCSs into mouse infarcted myocardium considerably improved left ventricular ejection fraction after 4 weeks. Furthermore, in addition to decreasing infarct size, this treatment increased anterior/septal ventricular wall thickness, and the capillary density similar to endogenous CSs. Results also revealed that iCSs and eCSs were engrafted into the myocardium and spontaneously differentiated into cardiomyocytes and endothelial cells *in vivo* 4 weeks after cell transplantation.[43]

This finding indicates that iCSs have therapeutic effects and can improve cardiac function following MI. Despite the use of pluripotency factors for the generation of iCSs, no tumor formation was reported 12 weeks after transplantation.

More recently, Panciera et al demonstrated that transient expression of exogenous YAP or TAZ, main downstream effectors of the Hippo signaling pathway, converts several differentiated mouse cell types to tissue-specific stem/progenitor cells.[46] The interesting point concerning this study is that YAP/TAZ induction converts each differentiated cell type into its corresponding stem/progenitor cell type with the memory of its tissue of origin. The induced progenitors produced by Panciera et al represented functional properties (i.e. self-renewal, differentiation ability, and *in vivo* regenerative potential) both *in vitro* and *in vivo*.[46] This finding shows that YAP/TAZ acts as a universal reprogramming factor capable of conversion of a differentiated cell type into its corresponding tissue-specific progenitor state.

## 5. Conclusions

Direct reprogramming is considered as a promising approach for human tissue regeneration. Lineage-restricted induced progenitors seem to possess superiority over terminally differentiated and mature induced cells for reconstruction of a diseased or damaged tissue. In this respect, it has been indicated that exogenous engineered cells should be at a progenitor state to be amenable to functional end organ engraftment and regeneration.[47] Induced progenitor cells can be generated *in vitro* and then transplanted to the target tissue. The other approach, which can circumvent hurdles of *in vitro* culture and preparation, is direct *in vivo* reprogramming entailing injection of progenitor reprogramming factors directly into the diseased tissue.

Although in an uncontrollable manner, transplanted iCPCs spontaneously differentiated into three cardiovascular cell types in cardiac environment. This shows that injured heart tissue provides an environment favorable and inductive to the cardiovascular three lineage differentiation of iCPCs. Thus, due to their compatibility with signals of the adult myocardium, iCPCs might be directly induced from cardiac resident fibroblasts *in vivo* by administration of cardiac reprogramming factors. However, the underlying mechanism of such differentiation is unknown and also aberrant differentiation of iCPCs could be a downside to this picture and should be addressed.

In addition to lineage-specific transcription factors, YAP/TAZ induction offers some ideas for *in vivo* reprogramming technology in various tissues. In this regard, *in situ* cardiac repair and regeneration directly using a universal single factor could advance *in vivo* cardiac reprogramming methods in terms of feasibility and convenience and will be a big step toward clinical regenerative medicine in the future.

Notably, current researches have been accomplished on small animal models; however, studies on large animal models are essential to investigate the effects of injected iCPCs and *in vivo* reprogramming in hearts, which are similar to the human heart. For instance, spontaneous three lineage differentiation of iCPCs might not occur in large animal models and probably in the human heart. Although here progenitor reprogramming has been discussed as a hope for heart regeneration either by injection of cultured iCPCs or direct *in vivo* reprogramming to iCPCs, it is expected that additional approaches such as tissue engineering are also required to fully restore the structure and function of the damaged tissue.

For *in vivo* reprogramming the use of viral vectors and engineered proteins may cause immunological reactions and inflammation that can reduce the efficacy of these approaches. Moreover, the inclusion of exogenous DNA into the host genome can lead to mutation, genomic alteration and dysregulation and also increase the risk of tumorigenicity and dysplasia especially in the use of pluripotency factors.[48,49] Although engineered proteins and synthetic RNAs remove safety concerns, they have a short half-life that reduces their effectiveness. To avoid safety and technical concerns regarding the use of viral vectors, synthetic RNAs and engineered proteins, thanks to their unique properties, small molecules that target specific signaling pathways and epigenetic processes offer powerful tools for engineering cell fate to the desired outcome. Some advantages of small molecules, including their cost-effectiveness, long half-life, and diversity in structure and function allow temporal and flexible regulation of signaling pathways, which can be applied effectively in tissue regeneration.[50,51] Suggestively, toward a chemical strategy, identification of small molecules capable of activation of endogenous YAP/TAZ or other key signaling pathways for induction of cardiac progenitor reprogramming is an interesting topic worthy of investigation. In this regard, *in vivo* progenitor reprogramming using a chemical-only approach will be a straightforward strategy for not only heart regeneration, but also for other tissues.

## Compliance with ethical standards

*Disclosure of potential conflicts of interest.*

The author declares that there is no competing interest.

*Research involving human participants and/or animal*

This article does not contain any studies with human or animal subjects performed by the any of the authors.

*Informed consent*

Not applicable.

*Funding*

This work was supported by Yazd Cardiovascular Research Center.

## Acknowledgements

The author would like to thank Dr. Russell C. Addis from Phelix Therapeutics for editing the manuscript.

## References

1. WHO. *Cardiovascular Diseases (CVDs). Fact Sheet N°317, 2015*; September 2016. Updated January 2015, Reviewed http://www.who.int/mediacentre/factsheets/fs317/en/.
2. Cleutjens JP, Blankesteijn WM, Daemen MJ, Smits JF. The infarcted myocardium: simply dead tissue, or a lively target for therapeutic interventions. *Cardiovasc Res*. 1999;44(2):232–241.
3. Jugdutt BI. Ventricular remodeling after infarction and the extracellular collagen matrix: when is enough enough? *Circulation*. 2003;108(11):1395–1403.
4. Snider P, Standley KN, Wang J, Azhar M, Doetschman T, Conway SJ. Origin of cardiac fibroblasts and the role of periostin. *Circ Res*. 2009;105(10):934–947.
5. Addis RC, Epstein JA. Induced regeneration-the progress and promise of direct reprogramming for heart repair. *Nat Med*. 2013;19(7):829–836.
6. Ebrahimi B. *In vivo* reprogramming for heart regeneration: a glance at efficiency, environmental impacts, challenges and future directions. *J Mol Cell Cardiol*. 2017;108:61–72.
7. Lalit PA, Salick MR, Nelson DO, et al. Lineage reprogramming of fibroblasts into proliferative induced cardiac progenitor cells by defined factors. *Cell Stem Cell*. 2016;18(3):354–367.
8. Zhang Y, Cao N, Huang Y, et al. Expandable cardiovascular progenitor cells reprogrammed from fibroblasts. *Cell Stem Cell*. 2016;18(3):368–381.
9. Lassar AB, Paterson BM, Weintraub H. Transfection of a DNA locus that mediates the conversion of 10T12 fibroblasts to myoblasts. *Cell*. 1986;47(5):649–656.
10. Davis RL, Weintraub H, Lassar AB. Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell*. 1987;51(6):987–1000.
11. Murry CE, Kay MA, Bartosek T, Hauschka SD, Schwartz SM. Muscle differentiation during repair of myocardial necrosis in rats via gene transfer with MyoD. *J Clin Investig*. 1996;98(10):2209–2217.
12. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126(4):663–676.
13. Xu J, Du Y, Deng H. Direct lineage reprogramming: strategies, mechanisms, and applications. *Cell Stem Cell*. 2015;16(2):119–134.
14. Ieda M, Fu JD, Delgado-Olguin P, et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell*. 2010;142(3):375–386.
15. Song K, Nam YJ, Luo X, et al. Heart repair by reprogramming non-myocytes with cardiac transcription factors. *Nature*. 2012;485(7400):599–604.
16. Jayawardena TM, Egemnazarov B, Finch EA, et al. MicroRNA-mediated *in vitro* and *in vivo* direct reprogramming of cardiac fibroblasts to cardiomyocytes. *Circ Res*. 2012;110(11):1465–1473.
17. Muraoka N, Yamakawa H, Miyamoto K, et al. MiR-133 promotes cardiac reprogramming by directly repressing Snai1 and silencing fibroblast signatures. *EMBO J*. 2014;33(14):1565–1581.
18. Efe JA, Hilcove S, Kim J, et al. Conversion of mouse fibroblasts into cardiomyocytes using a direct reprogramming strategy. *Nat Cell Biol*. 2011;13(3),215–U61.
19. Ebrahimi B. Engineering cell fate: spotlight on cell-activation and signaling-directed lineage conversion. *Tissue Cell*. 2016;48(5):475–487.
20. Wang H, Cao N, Spencer CI, et al. Small molecules enable cardiac reprogramming of mouse fibroblasts with a single factor, Oct4. *Cell Rep*. 2014;6(5):951–960.
21. Protze S, Khattak S, Poulet C, Lindemann D, Tanaka EM, Ravens U. A new approach to transcription factor screening for reprogramming of fibroblasts to cardiomyocyte-like cells. *J Mol Cell Cardiol*. 2012;53(3):323–332.
22. Addis RC, Ifkovits JL, Pinto F, et al. Optimization of direct fibroblast reprogramming to cardiomyocytes using calcium activity as a functional measure of success. *J Mol Cell Cardiol*. 2013;60:97–106.
23. Hirai H, Katoku-Kikyo N, Keirstead SA, Kikyo N. Accelerated direct reprogramming of fibroblasts into cardiomyocyte-like cells with the MyoD transactivation domain. *Cardiovasc Res*. 2013;100(1):105–113.
24. Ebrahimi B. Reprogramming barriers and enhancers: strategies to enhance the efficiency and kinetics of induced pluripotency. *Cell Regen*. 2015;4:10.
25. Ifkovits JL, Addis RC, Epstein JA, Gearhart JD. Inhibition of TGFβ signaling increases direct conversion of fibroblasts to induced cardiomyocytes. *PLoS One*. 2014;9(2), e89678.
26. Zhao Y, Londono P, Cao Y, et al. High-efficiency reprogramming of fibroblasts into cardiomyocytes requires suppression of pro-fibrotic signalling. *Nat Commun*. 2015;6:8243.
27. Yamakawa H, Muraoka N, Miyamoto K, et al. Fibroblast growth factors and vascular endothelial growth factor promote cardiac reprogramming under defined conditions. *Stem Cell Rep*. 2015;5(6):1128–1142.
28. Zhou H, Dickson ME, Kim MS, Bassel-Duby R, Olson EN. Akt1/protein kinase B enhances transcriptional reprogramming of fibroblasts to functional cardiomyocytes. *Proc Natl Acad Sci U S A*. 2015;112(38):11864–11869.
29. Abad M, Hashimoto H, Zhou H, et al. Notch inhibition enhances cardiac reprogramming by increasing MEF2C transcriptional activity. *Stem Cell Rep*. 2017;8(3):548–560.
30. Fu Y, Huang C, Xu X, et al. Direct reprogramming of mouse fibroblasts into cardiomyocytes with chemical cocktails. *Cell Res*. 2015;25(9):1013–1024.
31. Inagawa K, Miyamoto K, Yamakawa H, et al. Induction of cardiomyocyte-like cells in infarct hearts by gene transfer of Gata4, Mef2c, and Tbx5. *Circ Res*. 2012;111(9):1147–1156.
32. Mathison M, Gersch RP, Nasser A, et al. *In vivo* cardiac cellular reprogramming efficacy is enhanced by angiogenic preconditioning of the infarcted myocardium with vascular endothelial growth factor. *J Am Heart Assoc*. 2012;1(6),e005652.
33. Qian L, Huang Y, Spencer CI, et al. *In vivo* reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. *Nature*. 2012;485(7400):593–598.
34. Mathison M, Singh VP, Gersch RP, et al. "Triplet" polycistronic vectors encoding Gata4, Mef2c, and Tbx5 enhances postinfarct ventricular functional improvement compared with singlet vectors. *J Thorac Cardiovasc Surg*. 2014;148(4):1656–1664. e2.
35. Ma H, Wang L, Yin C, Liu J, Qian L. *In vivo* cardiac reprogramming using an optimal single polycistronic construct. *Cardiovasc Res*. 2015;108(2):217–219.
36. Jayawardena TM, Finch EA, Zhang L, et al. MicroRNA induced cardiac reprogramming *in vivo*: evidence for mature cardiac myocytes and improved cardiac function. *Circ Res*. 2015;116(3):418–424.
37. Wang L, Liu Z, Yin C, et al. Stoichiometry of Gata4, Mef2c, and Tbx5 influences the efficiency and quality of induced cardiac myocyte reprogramming. *Circ Res*. 2015;116(2):237–244.
38. Islas JF, Liu Y, Weng KC, et al. Transcription factors ETS2 and MESP1 transdifferentiate human dermal fibroblasts into cardiac progenitors. *Proc Natl Acad Sci U S A*. 2012;109(32):13016–13021.
39. Moretti A, Caron L, Nakano A, et al. Multipotent embryonic Isl1(+) progenitor cells lead to cardiac, smooth muscle, and endothelial cell diversification. *Cell*. 2006;127(6):1151–1165.
40. Kattman SJ, Huber TL, Keller GM. Multipotent flk-1+ cardiovascular progenitor cells give rise to the cardiomyocyte, endothelial, and vascular smooth muscle lineages. *Dev Cell*. 2006;11(5):723–732.
41. Li XH, Li Q, Jiang L, et al. Generation of functional human cardiac progenitor cells by high-efficiency protein transduction. *Stem Cells Transl Med*. 2015;4(12):1415–1424.
42. Xu J, Lian W, Li L, Huang Z. Generation of induced cardiac progenitor cells via somatic reprogramming. *Oncotarget*. 2017;8(17):29442–29457.
43. Xu JY, Lee YK, Ran X, et al. Generation of induced cardiospheres via reprogramming of skin fibroblasts for myocardial regeneration. *Stem Cell*. 2016;34(11):2693–2706.
44. Simpson DL, Mishra R, Sharma S, et al. A strong regenerative ability of cardiac stem cells derived from neonatal hearts. *Circulation*. 2012;126(11 suppl 1):S46–S53.
45. Ishigami S, Ohtsuki S, Tarui S, et al. Intracoronary autologous cardiac progenitor cell transfer in patients with hypoplastic left heart syndrome: the TICAP prospective phase 1 controlled trial. *Circ Res*. 2015;116(4):653–664.
46. Panciera T, Azzolin L, Fujimura A, et al. Induction of expandable tissue-specific stem/progenitor cells through transient expression of YAP/TAZ. *Cell Stem Cell*. 2016;19(6):725–737.
47. Morris SA, Cahan P, Li H, et al. Dissecting engineered cell types and enhancing cell fate conversion via CellNet. *Cell*. 2014;158(4):889–902.
48. Ma H, Morey R, O'Neil RC, et al. Abnormalities in human pluripotent cells due to reprogramming mechanisms. *Nature*. 2014;511(7508):177–183.
49. Lee AS, Tang C, Rao MS, Weissman IL, Wu JC. Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. *Nat Med*. 2013;19(8):998–1004.
50. Ebrahimi B. Chemicals as the sole transformers of cell fate. *Int J Stem Cells*. 2016;9(1):9–20.
51. Ebrahimi B. Chemical-only reprogramming to pluripotency. *Front Biol*. 2016;11(2):75–84.