

Detailed response to the Reviewers' comments

Reviewer #1: Regenerative potential of pluripotent stem cell-derived PDGFR α + cardiac lineage committed cells in infarcted myocardium This is an excellent paper describing in vivo utility of the authors earlier isolated progenitor population derived from embryonic stem cells. The authors provide extensive delineation of reparation derived from the progenitor population in an immunodeficient animal model of myocardial infarction.

Answer) We would like to express our deepest gratitude to you for taking the effort and time for reviewing our manuscript.

Reviewer #2: The manuscript reports about the role of cardiac progenitor cells derived from ESC (embryonic stem cells) in restoring cardiac function in a small animal (mice) model of cardiac infraction. The study is well designed, results correctly presented and interpreted. Below are point by point comments

Answer) First of all, we would like to express our deepest gratitude to you for taking the effort and time for reviewing our manuscript and providing us a thorough and constructive review.

Title

No mention here about the type of pluripotent stem cells used to obtaining cardiac progenitor. Is this because ESC are not obligatory fashionable nor ethically easy compared to iPSCs or is just in order to incite the reader to discover him/her self the origin of such cells?

Abstract

Structured and well organized, same mention here about keeping to denomination of PSCs derived cardiac progenitors. The reviewer does not pretend it is incorrect but rather that this denomination is nonspecific as they are more than one types of pluripotent stem cells out there. Do the authors have a specific reason not to mention the actual origin of these cardiac progenitor (here ESCs?) or do they consider their results apply to ALL types of pluripotent stem cell sources (including iPSCs?)

Answer) We fully agree with your important comments. Our group previously established a novel class of cells from PSCs – platelet-derived growth factor receptor- α (PDGFR α)⁺ CLCs—induced using a combination of four specific modulators (CsAYTE). In this study, we confirmed that CsAYTE induced PDGFR α ⁺ CLCs from not only mouse ESCs, but also mouse and human iPSCs (Sci Rep 2017; 7: 41840). Furthermore, recent large amount of studies demonstrated that both ESC and iPSC-derived cardiomyocytes have similar effect for cardiac regeneration. Therefore, we believe that iPSC-derived PDGFR α ⁺ CLCs also might have similar regenerative potential in infarcted myocardium. However, we could not perform the experiments in this study due to several technical issues (reporter cell line and etc.). Following your comments, we changed the PSCs to mouse ESCs in revised manuscript.

Introduction

Is concise and relevant to the topic. Do the authors consider their method of obtaining still proliferating but cardiomyocyte committed cells is useful somehow in deriving an algorithm for sorting other cell population?

Answer) These are also important points that were well taken by all the authors. Our group previously reported that CsAYTE mainly induced PDGFR α ⁺ CLCs and markedly reduced differentiation from mouse ESC-derived Flk1⁺ MPCs into CD144⁺CD31⁺ endothelial cells and CD41⁺ early hematopoietic cells to less than 1%. Furthermore, more than 95% of the sorted PDGFR α ⁺ CLCs spontaneously differentiated into cTnT⁺ and α MHC-GFP⁺ cardiomyocytes (Sci Rep 2017; 7: 41840). Therefore, we believe that method of obtaining PDGFR α ⁺ CLCs after CsAYTE treatment is useful in terms of early purification of cardiac lineage cells from PSCs.

What would be the meaning of introducing a paragraph about nonexistent cardiac progenitor markers?

Answer) So far, reported markers of cardiac progenitors are mostly transcription factors and intracellular proteins, not surface marker and we could not sort out these cells without reporter cell line. Therefore, we mentioned that “no definite surface

marker" in Introduction section.

Material and methods

Very well and thoroughly described

Results

Correctly presented and interpreted, an elegant proof of cardiac progenitor implantation within infarcted area and assessment of their morphological and functional significance compared to nontreated hearts. Can the authors consider in the future to quantitatively assess and compare engraftment and/or differentiation rate of cardiac progenitors after implantation?

Answer) These are also important points that were well taken by all the authors. As we already mentioned in Discussion section, it is very difficult to quantitatively assess and compare engraftment and/or differentiation rate of cardiac progenitors after implantation in small murine model due to technical issue. Further experiments using large animal models, such as swine or non-human primates, might be necessary for quantification of implanted cells.

Discussion

A very thorough argumentation on the benefits of using precommitted cardiac progenitor cells that retain expansion potential and apparently differentiate in vivo preferentially to cardiomyocytes and not to other lineages. However, without a quantitative assessment of comparative survival and engraftment rate it is really difficult to argue about such cell superiority for serving as therapeutic cells especially given the fact their morphological and functional effects compared to committed cells are similar. As very well presented in the discussion, long term cell survival is an issue (it is meritory authors have an extended observation period up to 60 days) therefore combined direct and paracrine effects are desirable. Maybe it would be good to assess as well endogenous cardiac progenitor recruitment (if any). The cell therapy delivery route in this study was direct myocardial injection immediately after infarction, very difficult to reproduce in clinical settings. How do

the authors consider a regional (intra-arterial delivery) and delayed (hours to days after infarction) are going to influence the results? Extremely good observation about the potential arrhythmogenic effect of injecting cells that retain their proliferative capability, this would be definitely a topic for further investigation.

Answer) Thank you very much for your constructive and important comments. Assessment of endogenous cardiac progenitor recruitment is very innovative and interesting idea for further investigation. Furthermore, we fully agree with your important comments in terms of cell delivery route. Although previous clinical trials of cardiac stem cell therapy by using adult stem cells used the intracoronary or intravenous route, there are no evidences of safety such as teratoma and cancer formation after PSC-derived cardiomyocytes injection via intracoronary or intravenous route to the best of our knowledge. Further experiments for demonstrating of safety and feasibility of PSC-derived cardiomyocytes injection via intracoronary or intravenous route should be performed.

Reviewer #3: Comments to the authors.

Answer) First of all, we would like to express our deepest gratitude to you for taking the effort and time for reviewing our manuscript and providing us a thorough and constructive review.

Major points

In this paper Hong et al. have evaluated the regenerative potential of PDGFR α ⁺ cardiac lineage committed cells (PDGFR α ⁺ CLCs) and α MHC⁺ cardiac myocytes (CMs) in a murine model of infarcted myocardium (MI). The authors found that these cells have improved the contractile function and structure of the infarcted heart upon implantation. It is appreciable that the authors have developed a unique method to induce PDGFR α ⁺ CLCs with chemicals (CsAYTE). They also have made the PDGFR α ⁺ CLCs and α MHC⁺ CMs tangible with td-Tomato and GFP, respectively. Although the present findings are interesting in terms of MI therapy, it remains unclear whether the implanted cells could survive and function more than two weeks. While the authors mention that "Both types of implanted cells persisted

up to 60 days after implantation (Figure 4C)", they did not address whether the implanted cells function properly.

Answer) These are very important points that were well taken by all the authors. Unfortunately, we could not perform the echocardiography at 60 days from implantation of cells. Following the reviewer's comments, we'll consider further studies investigating functional and histologic recovery after long-term period from implantation of PDGFR α ⁺ CLCs.

Besides these critiques, the paper did not address whether the use of reporter genes such as td-Tomato and GFP in ES cells would compromise the proper function of PDGFR α ⁺ CLCs and α MHC⁺ CMs in vivo.

Answer) We fully agree with your important comments. However, implanted cells expressing fluorescence like tdTomato or GFP by genetic modification are obliged to use for tracing the cells in the same species in vivo. To overcome this issue, implanted cells tagged fluorescence dye can be used, but this method also affect to the function of implanted cells and not feasible for observation of long-term engraftment.

Minor points

The text can be more succinct. When use %, please precise whether it is (v/v), (w/v), or (v/w).

Answer) Following the reviewer's comments, we modified in revised manuscript.

There are some typographical errors and the word "antigen recovery" is uncomprehensible.

Answer) Following the reviewer's comments, we changed "antigen recovery" into "antigen retrieval" in revised manuscript.