Peer Review File

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Reviewer A：

The authors of this well written manuscript provide a good review on cardiac regeneration, summarizing the methods, models and potential barriers to promote heart regeneration. Please find below my contribution:

A) Page 11 line 292. The topic "ShRNA-based Loss-of-Function Screen" is limited to ex vivo and provides small information about the Key methods to study heart regeneration. I recommend the authors include reporter animals like MADM (mosaic analysis with double markers) which have been used to assess cytokinesis events by lineage tracing. In addition, the authors could include qualitative and quantitative histological methods to accesses cardiomyocyte proliferation (PCNA, PHH3, BrdU, EdU…), associated with cardiomyocyte counting (by dissociation or stereological histology).

Reply: Thank you very much for your kind suggestions. We add "**4. Lineage tracing models:** Lineage tracing was developed to track proliferating cells and can be used to mark a number of cells at a specific time point [77]. Mosaic analysis with double markers (MADMs) is a powerful tool of lineage tracing to mark recombination events in both mitotic and postmitotic cells [78]. MADMs enables in vivo analysis of gene function with simultaneous labeling and gene knockout at a single-cell level and has been well applied in the studies of cardiac regeneration [79, 80]. Another stochastic model for lineage tracing is the rainbow reporter system, which works through fluorophore mediated cell labeling. Rainbow reporter system has been proven to be a useful lineage tracing tool in studying the cardiomyocyte proliferation during normal development as well as pathological conditions [81-84]." to the part of "Key Methods to Study Heart Regeneration", and delete "ShRNA-based Loss-of-Function Screen" (Page: 11, Line:298-307).

In addition, the content of "**5. Quantitative histological analysis of cardiomyocyte proliferation:** Quantitative histological method to accesses cardiomyocyte proliferation associated with cardiomyocyte counting is one of the critical technicians in the study of cardiac

proliferation. Nucleotide incorporation using 5-bromo-2'-deoxyuridine (BrdU) or 5-ethynyl-2-deoxyuridine (EdU) are widely acknowledged as a reliable measure of cell cycle reentry and proliferation [85-87]. By co-localization of mitotic marker pHH3 or S-phase marker PCNA with the cardiomyocyte marker myosin heavy chain, proliferated cardiomyocytes with incorporation of additional nucleotide can be visualized and quantified through immunofluorescent microscopy [88] ." also has been added. (Page: 11, Line: 300-307)

B) Page 8 line 212: "…which is different from M (IFN-gamma) or M (IL-4) phenotypes…" The sentence it unclear. Furthermore, I did not find any information about the distinct macrophages phenotypes in reference 58.

Reply: Thank you very much for your kind suggestion. We revised the sentence accordingly, now the sentence will be "Macrophage depletion in neonates did not influence cardiomyocyte proliferation with reduced cardiac function and angiogenesis after injury [58]. The authors further compared the immunophenotyping and gene expression profiling of cardiac macrophages from regenerating and non-regenerating hearts following MI. They found that macrophages from neonatal hearts have a unique polarization with no clear bias toward M1 or M2, while macrophages from post neonatal hearts have dramatically upregulated level of M2." (Page: 8, Line: 211-219)

Reviewer B

This manuscript reviews major concepts in the field of heart regeneration, highlighting studies describing cardiomyocyte renewal, barriers to heart regeneration, models to study heart regeneration, and methods to promote cardiac regeneration. The authors cover key concepts/papers adequately, and the paper is well-organized. There are numerous grammatical errors throughout; editing for English language grammar is strongly recommended.

Reply : Thank you very much for your reminder. This manuscript has been rechecked for grammatical errors and will be sent to extensive language editing.

Specific suggestions:

The phrase "Amending broken heart" in the title does not make sense (i.e. the word "amending"

does not seem appropriate here). Perhaps the authors meant, “Mending broken hearts” or “Mending a broken heart”?

Reply: Thank you for your kind suggestion. The title will be modified as “Mending a broken heart: targeting cardiomyocyte regeneration-a literature review”.

In Figure 1, different font sizes are used (i.e. polyploidization is a serif font while other boxes have a sans serif font). This should be uniform.

Reply: Thank you for your kind suggestion. Figure 1 and figure 2 have been revised carefully and now the front is uniform.

Reference 14 has been retracted, and this reference and its discussion should be omitted from this review (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 – retraction notice https://pubmed.ncbi.nlm.nih.gov/30582463/).

Reply: Thank you for your very important suggestion. All the contents related to reference 14 have been removed from the manuscript. (Page:4, line:89-93)

The following sentence on page 5 (1st sentence in the section entitled, “Potential Barriers to Heart Regeneration”) has circular logic: “The extremely limited regenerative potential of postnatal cardiomyocytes causes the loss of regeneration ability in mammalian cardiomyocytes.” (i.e. Low regenerative ability of cardiomyocytes causes low regenerative ability of cardiomyocytes.) This sentence should be reworded.

Reply: Thank you very much for your reminder. The sentence has been deleted. (Page: 5, line: 124-125)