nature portfolio

Peer Review File

Myc overexpression improves recovery from myocardial infarction associated to cardiomyocyte hyperplasia in the mouse heart

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This study investigated the role of Myc moderate overexpression in the hearts following ischemic injury in mice, focusing on its impact on cardiomyocyte division and regeneration. The authors performed the experiments following acute myocardial infarction in P7 and adult (8-12 weeks) mice. The authors claim that moderate overexpression of Myc in cardiomyocytes elicits a beneficial response after ischemic injury, mainly by promoting hyperplasia instead of hypertrophy via the stimulation of new cardiomyocytes. Overall, the authors provide descriptive in-vivo and in-vitro evidence to suggest that a heart with moderate Myc overexpression shows a reduced scar area and improved function and recovery following acute myocardial infarction, although, the mechanism underlying these effects remains poorly defined.

Major comments:
1. The authors report that Myc moderate overexpression led to less scarring and improved cardiac function. This improvement was shown in P7 mice, which differ from adults. Clarifying the model, age and context is required to avoid misinterpretation.
2. The claim that 'Myc mild overexpression promotes a regenerative response with no hypertrophy in adult hearts following ischemic injury' requires stronger evidence to substantiate the statement of regeneration. Specifically, regeneration in the heart typically implies a significant increase in cardiomyocyte (CM) proliferation. To support this claim, it would have been nice to add additional data, such as: Immunofluorescence staining (Cardiac sections derived from both WT (MHC-cre control) and MYC overexpression), with proliferation markers (e.g., Ki67, Edu, or PH3) to directly assess CM proliferation, in in-vivo and in-vitro (P1 or P7 cardiac culture) settings. Otherwise, the claims should fit the findings supported by the data.
3. I found some parts of the results section challenging to follow, as the organization and flow of results were not always clear. To enhance readability, I suggest structuring the results more systematically. For example, grouping related findings under clear subheadings or presenting the data in a chronological or thematic order might help. Additionally, summarizing key findings at the end of each subsection could provide a better sense of continuity and coherence. These adjustments could make the manuscript more accessible and engaging for readers.

Minor comments:
1. In Figure 3, the details for panel E are missing. Please include the relevant description in the figure legend.

Reviewer #2

(Remarks to the Author)
This study investigates the role of moderate Myc overexpression in promoting cardiac regeneration after myocardial infarction (MI) in mammalian hearts at P7 and in adult hearts. The authors found that moderate overexpression of Myc resulted in improved cardiac function and reduced scar formation after MI. In addition, the study shows a shift towards a hyperplastic phenotype, characterised by smaller cardiomyocyte size and increased heart size, in both P7 and adult hearts. When injured at P7, there was an increase in BrdU incorporation in mono-, bi- and multinucleated cardiomyocytes in Myc overexpressing mice. The author concluded that mononucleated CM give rise to de-novo cardiomyocytes and new bi-nucleated cardiomyocytes, which partially explain the phenotype and regeneration.

This study is significant because it identifies Myc as a potential target for inducing cardiac repair in non-regenerative adult hearts.

However, some questions remain.

The size of the nuclei in isolated BrdU+ cardiomyocytes looks different. What is the proportion of polyploid nuclei after Myc overexpression in mono- and binucleated cardiomyocytes?

Why don't the authors show similar BrdU data for the adult infarcts as for the P7 injury? Smaller CMs should also be indicative of CM proliferation. If you cannot provide similar data for the adult animals, please be more specific and add "neonatal" to the title and abstract.

Please do not use the term "progenitors" as this implies that mononucleated cardiomyocytes have a different phenotype compared to bi-nucleated CMs. This is very controversial. Do the authors have new data to support this term?

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
i am fine with the revision and would accept the paper

Reviewer #2

(Remarks to the Author)
All concerns have been addressed. Thank you.

Response to reviewers

We sincerely thank the reviewers for their valuable feedback and constructive comments, which have enhanced the quality of our manuscript.

Please find below the responses to each of the comments, addressed point by point:
Reviewers' comments:

Reviewer #1 (Remarks to the Author):

This study investigated the role of Myc moderate overexpression in the hearts following ischemic injury in mice, focusing on its impact on cardiomyocyte division and regeneration. The authors performed the experiments following acute myocardial infarction in P7 and adult (8-12 weeks) mice. The authors claim that moderate overexpression of Myc in cardiomyocytes elicits a beneficial response after ischemic injury, mainly by promoting hyperplasia instead of hypertrophy via the stimulation of new cardiomyocytes. Overall, the authors provide descriptive in-vivo and in-vitro evidence to suggest that a heart with moderate Myc overexpression shows a reduced scar area and improved function and recovery following acute myocardial infarction, although, the mechanism underlying these effects remains poorly defined.

Major comments:
1. The authors report that Myc moderate overexpression led to less scarring and improved cardiac function. This improvement was shown in P7 mice, which differ from adults. Clarifying the model, age and context is required to avoid misinterpretation.

We appreciate this comment, as it is indeed true that cardiomyocyte biology differs between adult and P7 hearts, despite both being classified as non-regenerative models. We have taken care to reflect this distinction throughout the text.

2. The claim that ‘Myc mild overexpression promotes a regenerative response with no hypertrophy in adult hearts following ischemic injury’ requires stronger evidence to substantiate the statement of regeneration. Specifically, regeneration in the heart typically implies a significant increase in cardiomyocyte (CM) proliferation. To support this claim, it would have been nice to add additional data, such as: Immunofluorescence staining (Cardiac sections derived from both WT (MHC-cre control) and MYC overexpression), with proliferation markers (e.g., Ki67, Edu, or PH3) to directly assess CM proliferation, in in-vivo and in-vitro (P1 or P7 cardiac culture) settings. Otherwise, the claims should fit the findings supported by the data.

We agree with the reviewer's comments and have thus included additional data following their suggestion. In figure 1, panel D, we have evaluated proliferation in P1 isolated cardiomyocytes from WT and MYC overexpressing mice, as proposed by the reviewer. Additionally, to provide *in vivo* data from adult hearts, we have quantified BrdU incorporation throughout a 3-week period in uninjured WT and Myc-overexpressing adult mice (10-12 weeks) (Figure 1 panel E). We also present data of cardiomyocyte ploidy in adult hearts –indirectly measured through determination of nuclear volume– in Figure 1F. The combined results of BrdU incorporation, ploidy and cardiomyocyte size (Figure 3) show that Myc increases cardiomyocyte proliferation in adult hearts.

3. I found some parts of the results section challenging to follow, as the organization and flow of results were not always clear. To enhance readability, I suggest structuring the results more systematically. For example, grouping related findings under clear subheadings or presenting the data in a chronological or thematic order might help. Additionally, summarizing key findings at the end of each subsection could provide a better sense of continuity and coherence. These adjustments could make the manuscript more accessible and engaging for readers.

We are grateful for this comment as we believe it has significantly improved the readability of our manuscript. We have included subheadings for each section, as well as conclusive remarks summarizing the major findings throughout the Results section. We hope these changes provide a more comprehensive read.

Minor comments:
1. In Figure 3, the details for panel E are missing. Please include the relevant description in the figure legend.

Details for Figure 3E have been added to the legend

Reviewer #2 (Remarks to the Author):

This study investigates the role of moderate Myc overexpression in promoting cardiac regeneration after myocardial infarction (MI) in mammalian hearts at P7 and in adult hearts. The authors found that moderate overexpression of Myc resulted in improved cardiac function and reduced scar formation after MI. In addition, the study shows a shift towards a hyperplastic phenotype, characterised by smaller cardiomyocyte size and increased heart size, in both P7 and adult hearts. When injured at P7, there was an increase in BrdU

incorporation in mono-, bi- and multinucleated cardiomyocytes in Myc overexpressing mice. The author concluded that mononucleated CM give rise to de-novo cardiomyocytes and new bi-nucleated cardiomyocytes, which partially explain the phenotype and regeneration.

This study is significant because it identifies Myc as a potential target for inducing cardiac repair in non-regenerative adult hearts.

However, some questions remain.

The size of the nuclei in isolated BrdU+ cardiomyocytes looks different. What is the proportion of polyploid nuclei after Myc overexpression in mono- and binucleated cardiomyocytes?

This question is very relevant because it addresses whether the increased DNA synthesis detected contributes to cardiomyocyte proliferation or polyploidization. We have analyzed the nuclear volume in both mononucleated and binucleated adult cardiomyocyte populations of Myc-overexpressing and WT adult hearts (Figure 1F). This analysis shows that the increase in BrdU incorporation detected both in bi- and mono-nucleated cardiomyocytes does not involve an increase in nuclear size. Rather, Myc-overexpressing cardiomyocytes show a tendency to reduction of nuclear size despite the increase in BrdU incorporation (Figure 1F). Given that nuclear size correlates with ploidy, these results indicate that Myc does not promote polyploidization but rather proliferation. Furthermore, given that bi-nucleated cardiomyocytes are largely unable to proliferate, the lack of increase in binuclear cardiomyocyte ploidy despite increased BrdU incorporation suggests that Myc overexpression also increases the generation of new binucleated cardiomyocytes from diploid cardiomyocytes.

Why don't the authors show similar BrdU data for the adult infarcts as for the P7 injury? Smaller CMs should also be indicative of CM proliferation. If you cannot provide similar data for the adult animals, please be more specific and add "neonatal" to the title and abstract.

Unfortunately, the adult experiments did not involve BrdU incorporation analysis and a new set of experiments would have to be performed to address this issue. We now provide evidence that even in the absence of injury adult cardiomyocytes proliferate more than WT cardiomyocytes (Figure 1E, F). We think that this observation, together with the limitation of cardiomyocyte hypertrophic response post-MI in adult hearts, strongly suggest that increased proliferation is involved in the response of Myc-overexpressing hearts in the adult heart. Nonetheless we have now revised the abstract, as suggested by the reviewer. We think that the title, as it stands, does not specifically refer to the adult heart and including “non-regenerative postnatal” would complicate it and suggest that we did not address the adult heart at all, therefore, we suggest to maintain the title as it is.

Please do not use the term "progenitors" as this implies that mononucleated cardiomyocytes have a different phenotype compared to bi-nucleated CMs. This is very controversial. Do the authors have new data to support this term?

Our analysis does not involve a phenotypic characterization of mono-nucleated vs bi-nucleated cardiomyocytes. The term “progenitor” is used here from a functional perspective, in the sense that bi-nucleated cardiomyocytes derive from mono-nucleated ones. Nonetheless, we understand that this term can be controversial and subject to diverse interpretations, therefore, we accepted the suggestion of the reviewer and eliminated this term.