Peer-Review Record

Cardiovascular aging: from cellular and molecular changes to therapeutic interventions

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by Angeliki Vakka, Junco S. Warren, Konstantinos Drosatos

Received: 28 Feb 2023 **| First Decision:** 21 Mar 2023 **| Revised:** 6 Apr 2023 **| Accepted:** 26 Apr 2023 **| Published:** 4 May 2023

Academic Editor: Ali J. Marian

Reviewer 1: Anonymous

Reviewer 2: Anonymous

Reviewer 3: Anonymous

Round 1

Reviewer 1 Report

The review article is timely, and the topic is interesting. The reviewer is very enthusiastic about the publication of this manuscript, which casts a huge net and covers lots of topics. Naturally, some of the topics have not been dealt with adequately (breadth > depth). The reviewer offers several suggestions to the authors as follows:

The reviewer recognizes that the first several sections are meant to be introductory material (up to cellular complications ….). They lack scientific depth and are not much informative. For example, 5 lines on clinical aspects of vascular aging do not do justice to this topic (or to the authors). This reviewer suggests deleting those sections as they are not up to par with the rest of the manuscript. Alternatively, the authors might wish to improve the scientific content of these sections. Another option would be to insert the clinical paragraph at the beginning of each section on cellular changes.

Despite the title, the review article does not focus on cell signaling pathways. It provides rather a broad discussion of molecular changes including signaling molecules that are associated with aging. The authors might wish to revise the title.

The structure and organization of the review are somewhat unclear. For example, the section on oxidative stress is broad and includes IFG1 signaling as well. The reviewer recognizes that the pathways are connected but reorganization into specific sections would enhance the clarity of the changes. This is particularly important for the IGF1 pathway, which has a well-established role in aging.

Parts of the review are too brief and general. Such sections should be expanded. For example, in lines 277-279, the section covers a large territory but is summarized in a single sentence.

There is also considerable interest in D-galactose and aging, involving multiple mechanisms. The reviewer suggests including a brief discussion about D-galactose.

As for the M1/M2 macrophages, it is now evident this is a cell culture phenotype, and such dichotomy does not exist *in vivo* and humans.

Lines 373-375: on genetic ablation of Tp53 and elimination of senescence but worsening of fibrosis. However, earlier it was stated that fibrosis is a hallmark of aging. It seems contradictory and might require expansion to explain.

On telomere, the authors might wish to explain that telomere length in mice and humans differ significantly. Consequently, changes in the telomere length upon aging differ between the two.

When describing the animal models of aging, the reviewer suggests expanding on the biological functions of the main gene/protein involved. This will help the readers to better appreciate the involved mechanisms.

In Table 1, the reviewer suggests including the underlying defect responsible for aging in each model.

Figure 1. in the pie chart, section fibrosis, only angiotensin is listed. Surely, it is not the sole factor.

Minutiae
Some of the topics in the sections discussed in the present paper have already been reviewed by others and recently published in the *JCA*, as listed on the Journal webpage. The authors might wish to shorten such sections and simply refer to the recent review articles in the *JCA* (lifestyle and exercise for example).

Line 54: “increased diameter of the left atrium” perhaps the authors should change it to an increase in the left atrial size.

Lines 84-87. Regarding cardiomyocyte hypertrophy with aging, the cited article does not directly assess cardiomyocyte hypertrophy. It would be preferable to provide direct (original research articles) that show cardiomyocyte hypertrophy with aging.

Line 245. Please check the reference format.

Line 352. Please check the reference format.

Author Response

The review article is timely, and the topic is interesting. The reviewer is very enthusiastic about the publication of this manuscript, which casts a huge net and covers lots of topics. Naturally, some of the topics have not been dealt with adequately (breadth > depth). The reviewer offers several suggestions to the authors as follows:

The reviewer recognizes that the first several sections are meant to be introductory material (up to cellular complications ….). They lack scientific depth and are not much informative. For example, 5 lines on clinical aspects of vascular aging do not do justice to this topic (or to the authors). This reviewer suggests deleting those sections as they are not up to par with the rest of the manuscript. Alternatively, the authors might wish to improve the scientific content of these sections. Another option would be to insert the clinical paragraph at the beginning of each section on cellular changes.

We are pleased for the enthusiasm that the reviewer expresses about our article, and we are thankful for the useful suggestions. We hope that our changes have addressed all issues satisfactorily.

As we anticipate that this article will be of interest for people with medical background, as well as that readers with basic sciences background would like to be informed about some of the clinical aspects of cardiovascular aging, instead of deleting these sections, we expanded the vascular aging topic (lines 65-74).

Despite the title, the review article does not focus on cell signaling pathways. It provides rather a broad discussion of molecular changes including signaling molecules that are associated with aging. The authors might wish to revise the title.

This is indeed a broad topic to cover all aspects within a review article. The reviewer is right that we did not cover signaling pathways in great depth. In accordance with the reviewer's suggestion, we changed the title, which now reads "Cardiovascular aging: From molecular and cellular changes to therapeutic interventions". We hope that the revised title reflects the content of the article more accurately.

The structure and organization of the review are somewhat unclear. For example, the section on oxidative stress is broad and includes IFG1 signaling as well. The reviewer recognizes that the pathways are connected but reorganization into specific sections would enhance the clarity of the changes. This is particularly important for the IGF1 pathway, which has a well-established role in aging.

We regret for the unclear layout of our article in the original manuscript. The oxidate stress section is now divided in pro-oxidant pathways and anti-oxidant mechanisms sub-sections. Moreover, we moved the IGF-1 and the intracellular calcium handling parts to the metabolism section, which now reads "Aging-related alterations in metabolic homeostasis". We hope that these modifications have improved clarity and flow of the article.

Parts of the review are too brief and general. Such sections should be expanded. For example, in lines 277-279, the section covers a large territory but is summarized in a single sentence.

In hope that our perception about this comment aligns with the reviewer's thoughts, we have added new content in the inflammation section (lines 340-347). The first sentence about inflammaging (lines 312-315 in the revised manuscript), aims to serve as the introduction of the entire section. To avoid misinterpretation that this sentence is supposed to stand alone, we have incorporated it in the paragraph that follows.

There is also considerable interest in D-galactose and aging, involving multiple mechanisms. The reviewer suggests including a brief discussion about D-galactose.

Along the lines of the reviewer's suggestion, in addition to the existing references to the association of D-galactose with aging we have added a paragraph about the D-galactose model of aging (lines 449-457).

As for the M1/M2 macrophages, it is now evident this is a cell culture phenotype, and such dichotomy does not exist in vivo and humans.

The reviewer is right in proposing to omit this controversial term, which we did in the revised manuscript.

Lines 373-375: on genetic ablation of Tp53 and elimination of senescence but worsening of fibrosis. However, earlier it was stated that fibrosis is a hallmark of aging. It seems contradictory and might require expansion to explain.

The following explanation has been added in this section: "However, genetic ablation of p53 and p16(INK4a) (Trp53-/-Cdkn2a-/- mice) eliminates senescence but it exacerbates fibrosis with pressure overload, resulting in severe cardiac dysfunction (121). Furthermore, cardiac-specific induction of senescence in the same study lowered perivascular fibrosis, which contradicts findings that correlate fibrosis with cardiac aging. This finding indicates potential protective effects of isolated cellular senescence against fibrosis in a young heart as opposed to an aged heart that cellular senescence is extensive".

On telomere, the authors might wish to explain that telomere length in mice and humans differ significantly. Consequently, changes in the telomere length upon aging differ between the two.

A relevant statement has been added in the "Telomere dysfunction" section (lines 414-418).

When describing the animal models of aging, the reviewer suggests expanding on the biological functions of the main gene/protein involved. This will help the readers to better appreciate the involved mechanisms.

This section is now expanded along the lines of the reviewer's suggestions (lines 449-477).

In Table 1, the reviewer suggests including the underlying defect responsible for aging in each model.

An additional column designated “relevance to aging” has been added in Table 1.

Figure 1. in the pie chart, section fibrosis, only angiotensin is listed. Surely, it is not the sole factor.

Figure 1 is now enriched with more factors that contribute in fibrosis or compromise autophagy.

Minutiae
Some of the topics in the sections discussed in the present paper have already been reviewed by others and recently published in the *JCA*, as listed on the Journal webpage. The authors might wish to shorten such sections and simply refer to the recent review articles in the *JCA* (lifestyle and exercise for example).

The exercise section was shortened slightly and two previously published JCA review article (Clayton ZS et al. and Elhelaly W and Sadek H) are now cited. However, although the Clayton ZS review focuses on exercise and calorie restriction, the context and the organization of that review differ from our article. Therefore, we performed minor changes and we hope that the reviewer is now pleased with how this section flows.

Line 54: “increased diameter of the left atrium” perhaps the authors should change it to an increase in the left atrial size.

This part has been omitted from the revised version of the manuscript.

Lines 84-87. Regarding cardiomyocyte hypertrophy with aging, the cited article does not directly assess cardiomyocyte hypertrophy. It would be preferable to provide direct (original research articles) that show cardiomyocyte hypertrophy with aging.

Original articles (Olivetti G et al. and Guideri G et al.) are now cited.

Line 245. Please check the reference format.

Checked and corrected.

Line 352. Please check the reference format.

Checked and corrected.

Reviewer 2 Report

The authors of this manuscript have chosen an interesting topic that encompasses different signaling components that are affect in cardiac aging. Overall, several aspects of this manuscript are informative and provide a good overview. However, the manuscript can be improved by providing more specifics and making it focused to cell signaling pathways in aging.

Figure 1 can be modified or split into 3 parts that provide a more detailed view of each signaling component. For example, KLOTHO and/or AMPK are shown to affect cardiovascular aging with multiple downstream effectors not shown in the current figure.

The section on the role of metabolic signaling in aging is not integrated with the rest of the manuscript and can be removed to focus on the key signaling changes in aging.

The following subtitle "CELLULAR COMPLICATIONS IN CARDIOVASCULAR AGING" is ambiguous and can be modified with "Dysregulation of cell signaling in cardiovascular aging" or something similar to make it more meaningful.

The data pertaining to the role of NOX4-CaMKII-RYR2 is rather correlative (reference 41) and therefore, the authors conclusion that the results "established causality" is not consistent with the findings and should be modified to reflect the correlative nature of the findings.

Conclusions are quite long and redundant and can be formulated more concisely.

Authors should use either the human or mouse gene symbol when describing them in general terms. For specific mouse or human experiments, the appropriate symbols should be used. For example, Arg-II should be ARG2 and henceforth for others.

Minor changes:

Line 151: ROS-induced mitochondrial ROS production should be modified to ROS-induced mitochondrial ROS release (RIRR).

Line 155: Please change "NO" to nitric oxide.

Line 161: Please define SRT1720.

Line 190: Please change "Klotho acts as incurs" to "Klotho incurs".

Line 352: Please delete the following "[(Sheng Ye at al., 2015)".

Line 369: please provide an appropriate reference(s) to support the following statement "Fibroblast activation and proliferation, along with hypertrophy of myocytes, make up for myocyte loss due to apoptosis and necrosis".

Several references are not formatted correctly and should be re assessed.

Author Response

The authors of this manuscript have chosen an interesting topic that encompasses different signaling components that are affect in cardiac aging. Overall, several aspects of this manuscript are informative and provide a good overview. However, the manuscript can be improved by providing more specifics and making it focused to cell signaling pathways in aging.

We thank the reviewer for the positive comments. We hope that the changes we made have improved the content of our manuscript.

Figure 1 can be modified or split into 3 parts that provide a more detailed view of each signaling component. For example, KLOTHO and/or AMPK are shown to affect cardiovascular aging with multiple downstream effectors not shown in the current figure.

Figure 1 has been enriched with more factors that contribute to aging. In addition, Klotho is now included in the inflammation section as well.

The section on the role of metabolic signaling in aging is not integrated with the rest of the manuscript and can be removed to focus on the key signaling changes in aging.

We consider the metabolic component of aging as one of the main avenues of the pathophysiology of the disease. We did not invest a large part of our article on this topic as our group has previously published two review articles on the topic and there might be significant overlap. To improve integration of this section in the rest of the manuscript, we restructured this part, including the title that now reads "Aging-related alterations in metabolic homeostasis", by moving the IGF1 and mitochondrial dysfunction parts in that.

The following subtitle "CELLULAR COMPLICATIONS IN CARDIOVASCULAR AGING" is ambiguous and can be modified with "Dysregulation of cell signaling in cardiovascular aging" or something similar to make it more meaningful.

The title of this section now reads "CELLULAR AND MOLECULAR PROCESSES IN CARDIOVASCULAR AGING". We hope that this title is more relevant to the content of the respective section.

The data pertaining to the role of NOX4-CaMKII-RYR2 is rather correlative (reference 41) and therefore, the authors conclusion that the results "established causality" is not consistent with the findings and should be modified to reflect the correlative nature of the findings.

We modified the relevant sentence which now reads "implied causality".

Conclusions are quite long and redundant and can be formulated more concisely.

The “Epilogue-Conclusions” section has been shortened and we hope that it is more succinct now.

Authors should use either the human or mouse gene symbol when describing them in general terms. For specific mouse or human experiments, the appropriate symbols should be used. For example, Arg-II should be ARG2 and henceforth for others.

We updated gene nomenclature in accordance with the Genome Browser for mouse and human genes.

Minor changes:

Line 151: ROS-induced mitochondrial ROS production should be modified to ROS-induced mitochondrial ROS release (RIRR).

This has been modified as suggested.

Line 155: Please change “NO” to nitric oxide.

This has been modified as suggested.

Line 161: Please define SRT1720.

This sentence now reads “Activation of SIRT1 with SRT1720, a specific SIRT1 activator, …”

Line 190: Please change “Klotho acts as incurs” to “Klotho incurs”.

This has been modified as suggested.

Line 352: Please delete the following “[(Sheng Ye at al., 2015)”.

This has been deleted as suggested.

Line 369: please provide an appropriate reference(s) to support the following statement “Fibroblast activation and proliferation, along with hypertrophy of myocytes, make up for myocyte loss due to apoptosis and necrosis”.

Four references have been added for this statement in the revised manuscript.

Several references are not formatted correctly and should be re assessed.

This has been corrected.

Reviewer 3 Report

Excellent review article.
The author needs to double check the manuscript for typos, grammatical errors as well as proper description of the abbreviations.

In Fig1, for a better illustration of the pathways in cardiovascular aging, the author should include more details under four pathways shown, particularly fibrosis and impaired autophagy, as described in the text.

Author Response

Excellent review article.

The author needs to double check the manuscript for typos, grammatical errors as well as proper description of the abbreviations.

We are pleased that the reviewer found our article to be excellent. We have checked the manuscript thoroughly for typos and grammatical errors and to the best of our understanding we have corrected all mistakes.

In Fig1, for a better illustration of the pathways in cardiovascular aging, the author should include more details under four pathways shown, particularly fibrosis and impaired autophagy, as described in the text.

Fig 1 has been enriched with more proteins that contribute to aging-related fibrosis and impairment of authophagy.