Peer-Review Record

Targeting vascular senescence in cardiovascular disease with aging

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by Shelby A. Hall, Lisa A. Lesniewski

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Academic Editor: Ali J. Marian

Reviewer 1: Anonymous

Reviewer 2: Anonymous

Round 1

Reviewer 1 Report

Thank you very much for the opportunity to review this manuscript. Drs. Hall and Lesniewski discuss targeting of senescence in the cardiovascular system. My comments are as follows:

This is an excellent and timely review. However, it is too dense and should be expanded to enhance the clarity of the various topics that are discussed. To an un-initiated reader, several sections might not be clear and they might miss the points of the article.

The main focus of the article is on vascular (endothelial) cell senescence which is fine. However, it does not quite fit with the title of the article as the reader would expect some discussion in other cell types as well. The easiest approach would be to change the title but this reviewer's preference would be to expand the review to include additional cell types, particularly cardiac myocytes.

The authors define cellular senescence as the arrest of cell division in response to the activation of tumor suppressors. The reviewer accepts the definition. Nevertheless, the reviewer wonders whether this definition applies to non-proliferating cells such as cardiac myocytes. Said otherwise, how to define senescence in the non-replicating cells such as cardiac myocytes? Is the TP53 pathway activated in the non-replicating cells that undergo senescence?

Concerning the above, the authors might wish to expand on whether the non-replicating cells also express SASP.

Likewise, are the genes encoding SASP the targets of the TP53 pathway? or other transcriptional regulators are also involved? FOXO TFs are come to mind. (of course, a number of them are targets of NFkB1 but how what is the link between TP53 activation and NFkB1)

The authors state that several SASPs are targets of NFkB, C/EBPbeta, and GATA4. It seems that a couple of statements to link activations of these transcriptional regulators in senescence, particularly senescence as defined by activation of cell cycle regulators and TP53 would be informative.

The paragraph starting with: "When a cell enters senescence, it can rewire …..", is a very dense paragraph with lots of data. For example, it is unclear how the CGAS/STING pathway links to the rest of the molecules that are mentioned. The reviewer suggests expanding this very important but very dense section to explain the salient points.

The reviewer also suggests expanding the section on mitochondria (page 5 of the PDF).

The reviewer suggests including the nomenclature for the cell cycle arrest markers which are more informative as many of the original descriptions of these molecules (including TP53 itself) were based on their molecular weights (typically on PAGE). For example, CDKN1A, CDKN2A and CKDN2B could be listed next to p21, p14(INK4A)/p14(ARF), and p15(INK4b), etc.

When discussing senescence in the endothelial cells, it might be informative to briefly mention the functional heterogeneity of these cells and whether cells in various territories senesce differently.

The reviewer suggests expanding on the discussion on how targeting a very small percentage of the cells that show evidence of senescence could impart functional effects at the organ level. The typical response might be it is the elimination or reduction of SASP that imparts the beneficial effects. However, the reviewer suggests that none of the known SASP imparts a large effect size on cardiac or vascular function. Perhaps, a combination of them might do, as the effect of each SASP is modest in size and mediated in the long term. It seems to this reviewer that many investigators are reporting beneficial short-term effects, which seem to be disproportionate to the elimination of a small number of cells in an organ or reduction of expression of the SASP. The authors might wish to expand and elaborate.

Author Response

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The main focus of the article is on vascular (endothelial) cell senescence which is fine. However, it does not quite fit with the title of the article as the reader would expect some discussion in other cell types as well. The easiest approach would be to change the title but this reviewer's preference would be to expand the review to include additional cell types, particularly cardiac myocytes.

Because the focus is on vascular cell senescence, we are changing the title to "Targeting Vascular Senescence in Cardiovascular Disease with Aging". The discussion of other cell types such as cardiomyocytes would need additional background and understanding and would take the focus off the main topic of endothelial and vascular smooth muscle cell senescence.

The authors define cellular senescence as the arrest of cell division in response to the activation of tumor suppressors. The reviewer accepts the definition. Nevertheless, the reviewer wonders whether this definition applies to non-proliferating cells such as cardiac myocytes. Said otherwise, how to define senescence in the non-replicating cells such as cardiac myocytes? Is the TP53 pathway activated in the non-replicating cells that undergo senescence?

Non-replicating cells can become senescent through the stress-induced senescence pathways. This was added.

Concerning the above, the authors might wish to expand on whether the non-replicating cells also express SASP.

Likewise, are the genes encoding SASP the targets of the TP53 pathway? or other transcriptional regulators are also involved? FOXO TFs are come to mind. (of course, a number of them are targets of NFkB1 but how what is the link between TP53 activation and NFkB1)

Both FOXO4 and p53 mediated transcriptional activation of p21 leads to the induction of cellular senescence.

The authors state that several SASPs are targets of NFkB, C/EBPbeta, and GATA4. It seems that a couple of statements to link activations of these transcriptional regulators in senescence, particularly senescence as defined by activation of cell cycle regulators and TP53 would be informative.

Statements describing targets of SASP listed were added.

The paragraph starting with: "When a cell enters senescence, it can rewire …..", is a very dense paragraph with lots of data. For example, it is unclear how the CGAS/STING pathway links to the rest of the molecules that are mentioned. The reviewer suggests expanding this very important but very dense section to explain the salient points.

More information regarding the CGAS/STING pathway was added.

The reviewer also suggests expanding the section on mitochondria (page 5 of the PDF).

More information regarding mitochondria and senescence was added.

The reviewer suggests including the nomenclature for the cell cycle arrest markers which are more informative as many of the original descriptions of these molecules (including TP53 itself) were based on their molecular weights (typically on PAGE). For example, CDKN1A, CDKN2A and CKDN2B could be listed next to p21, p14(INK4A)/p14(ARF), and p15(INK4b), etc.

When discussing senescence in the endothelial cells, it might be informative to briefly mention the functional heterogeneity of these cells and whether cells in various territories senesce differently.

The functional heterogeneity of the endothelial cells and information regarding ECs was included.

The reviewer suggests expanding on the discussion on how targeting a very small percentage of the cells that show evidence of senescence could impart functional effects at the organ level. The typical response might be it is the elimination or reduction of SASP that imparts the beneficial effects. However, the reviewer suggests that none of the known SASP imparts a large effect size on cardiac or vascular function. Perhaps, a combination of them might do, as the effect of each SASP is modest in size and mediated in the long term. It seems to this reviewer that many investigators are reporting beneficial short-term effects, which seem to be disproportionate to the elimination of a small number of cells in an organ or reduction of expression of the SASP. The authors might wish to expand and elaborate.

A sentence addressing this comment was added to clarify that reducing senescence decreases the host SASP factors leading to an overall decrease in inflammation alleviating age-related diseases. Although it is only a small portion of cells being reduced, these cells accumulate within tissues and can act on neighboring cells through paracrine signaling causing them to become senescent as well.

Reviewer 2 Report

Shelby Hall et al. summarized the therapy targeting cellular senescence in cardiovascular diseases showing the feature of cellular senescence in cardiovascular diseases and the evidence of senolytics, elimination of senescent cells. Unfortunately, throughout this manuscript there are so many inappropriate references and key references are missing in multiple parts. I will provide a few examples below.

There are several references which are not relevant to the sentences.
The reference 35 is about arterial stiffness in healthy subject and not relevant to “inflammation”, “arterial dysfunction and disease”.

The citation 38 is about endothelial dependent vascular dilatation. In this paper, there is no data about "endothelin-1, Vcam-1 or Icam-1". The reference 39 is about vascular smooth muscle cells not about endothelial cells.
The reference 40 is editorial article. Please change to original article.
The citations listed (44, 45) are not relevant to senescence and atherosclerosis.

The cited papers are largely outdated even though cellular senescence and CVD have been well studied.
The reference 1 is published in 2017. Is there any updated reference instead of reference 1? There are also multiple studies about aging and CVDs, or endothelial functions. The references 2-5 and 34-39 is also considered to be updated, ideally citing primary research literature (not a review).

Key references are missing in multiple part throughout the manuscript.
The reference 11 is not focused on SASP factors and key references are not included (e.g., PMID: 31945054, PMID: 28844647, PMID: 28699239, PMID: 32554926)
The reference 14 is about the anti-apoptotic pathway in cancer cells, not in senescent cells and key reference is not cited (PMID: 26711051, PMID: 26657143).
Many key studies that should have been included are missing (Atherosclerosis PMID: 34142149, PMID: 31636264, PMID: 31799499, PMID: 33446552, PMID: 26116697, PMID: 37117524, vascular function PMID: 26864908).

It is a bit difficult to read at times because it is not clear whether the sentences are related with CVDs and endothelial functions, or general evidence of cellular senescence. For example, there are not relevant to the endothelial cells or CVDs in reference 27 (review about mitochondrial dysfunction and cell senescence) and 29 (in bone marrow mesenchymal stem cells), whereas reference 28 is about aortic stiffness. The discussion mixing these leads a false perception. I recommend that authors should use more references relevant to endothelial cells, vascular dysfunction, or CVDs, and note clearly like "in endothelial cells", or "in mesenchymal stem cells, and it might be effective to endothelial cells". It is also not clear the authors want to focus on endothelial cells or vascular smooth muscle cells in the section of atherosclerosis. The latter half of this section is about smooth muscle cells. If they don't need to focus on endothelial cells, they should add the section about vascular smooth muscle cells as well as endothelial cells.

In Hallmarks of cellular senescence section, SA-beta-Gal staining is one of the commonly used assays to detect cellular senescence and related with the loss of proteostasis (PMID: 36599349, PMID: 11927518).
The urinary α-Klotho was also identified as a senescence marker which may be a useful test for clinical trials.
In addition to DPP4, there are several newly identified markers of cellular senescence including PD-L1(PMID: 36323784), uPAR(PMID: 32555459), GPNMB(PMID: 37117524), CD153(PMID: 32424156).

In the paragraph of endothelial dysfunction, most of the evidence are supported by in vitro study. The paper using endothelial specific p53 knockout mice might be considered as a in vivo study (PMID: 30790589).

The paragraph about senolytics and ferroptosis is interesting, but it is too much focused in spite of the small amount of evidence. As the authors mentioned like “information on their role in the context of aging and senescence is deficient.” in the manuscript, most of the experiments were done by in vitro or ex vivo and there is no evidence in the context of CVDs, vascular function or endothelial cells. I wonder if this paragraph is necessary in the review tiled “Targeting Senescence in Cardiovascular Disease with Aging”.

“ in clinical settings” should be added to the last paragraph “Currently, there is not enough research on the use and treatment of senolytic therapy in cardiovascular diseases.”

A review should summarize literature in a critical manner. Thre is evidence that elimination of senescent pulmonary endothelial cells by ABT263 or FOXO4-DRI interventions may worsen pulmonary hemodynamics (PMID: 36515093). More studies about long-term effects or side-effects should be done.

“toward higher levels of ABT42, a biomarker that is inversely related to Alzheimer’s disease diagnosis. 65”
ABT42 should be typo. It is AB42. This reference 65 is preprint. The correct reference is PMID: 37679434.

The name of the journal should be bold in the reference 40.

Author Response

Shelby Hall et al. summarized the therapy targeting cellular senescence in cardiovascular diseases showing the feature of cellular senescence in cardiovascular diseases and the evidence of senolytics, elimination of senescent cells. Unfortunately, throughout this manuscript there are so many inappropriate references and key references are missing in multiple parts. I will provide a few examples below.

There are several references which are not relevant to the sentences.
The reference 35 is about arterial stiffness in healthy subject and not relevant to “inflammation”, “arterial dysfunction and disease”.

Changed to reference: Freeman, B. D., Machado, F. S., Tanowitz, H. B. & Desruisseaux, M. S. Endothelin-1 and its role in the pathogenesis of infectious diseases. Life Sci. 118, 110 – 119 (2014). AND 10.3390/biomedicines9040328 and Chakraborty, S.; Hu, S.-Y.; Wu, S.-H.; Karmenyan, A.; Chiou, A. The Interaction Affinity between Vascular Cell Adhesion Molecule-1 (VCAM-1) and Very Late Antigen-4 (VLA-4) Analyzed by Quantitative FRET. PLoS ONE 2015, 10, e0121399.

The citation 38 is about endothelial dependent vascular dilatation. In this paper, there is no data about "endothelin-1, Vcam-1 or Icam-1". The reference 39 is about vascular smooth muscle cells not about endothelial cells.
The reference 40 is editorial article. Please change to original article.

Changed to local matrix metalloproteinase 9 level determines early clinical presenation of ST-segment-elevation myocardial infarction Tsuyoshi nishiguchi.

The citations listed (44, 45) are not relevant to senescence and atherosclerosis.

Citations were removed and citations relevant to senescence and atherosclerosis were included.

The cited papers are largely outdated even though cellular senescence and CVD have been well studied.
The reference 1 is published in 2017. Is there any updated reference instead of reference 1?

Added reference: PMID:34220975.

There are also multiple studies about aging and CVDs, or endothelial functions. The references 2-5 and 34-39 is also considered to be updated, ideally citing primary research literature (not a review).

Changed refs 2-5 to (DOI: 10.2174/092986707779313354 PMID: 15123572, PMID:19465546).

Key references are missing in multiple part throughout the manuscript.
The reference 11 is not focused on SASP factors and key references are not included (e.g., PMID: 31945054, PMID: 28844647, PMID: 28699239, PMID: 32554926)

Included key references about SASP: PMID: 31945054, PMID: 28844647, PMID: 28699239, PMID: 32554926)

The reference 14 is about the anti-apoptotic pathway in cancer cells, not in senescent cells and key reference is not cited (PMID: 26711051, PMID: 26657143).

These references were added.

Many key studies that should have been included are missing (Atherosclerosis PMID: 34142149, PMID: 31636264, PMID: 31799499, PMID: 33446552, PMID: 26116697, PMID: 37117524, vascular function PMID: 26864908).

Key studies relevant to the review paper were added: PMID: 26864908 PMID: 26116697, PMID: 37117524 PMID: 34142149, PMID: 31636264

It is a bit difficult to read at times because it is not clear whether the sentences are related with CVDs and endothelial functions, or general evidence of cellular senescence. For example, there are not relevant to the endothelial cells or CVDs in reference 27 (review about mitochondrial dysfunction and cell senescence) and 29 (in bone marrow mesenchymal stem cells), whereas reference 28 is about aortic stiffness. The discussion mixing these leads a false perception. I recommend that authors should use more references relevant to endothelial cells, vascular dysfunction, or CVDs, and note clearly like "in endothelial cells", or "in mesenchymal stem cells, and it might be effective to endothelial cells". It is also not clear the authors want to focus on endothelial cells or vascular smooth muscle cells in the section of atherosclerosis. The latter half of this section is about smooth muscle cells. If they don't need to focus on endothelial cells, they should add the section about vascular smooth muscle cells as well as endothelial cells.

More information about endothelial cells was added and information connecting ECs to VSMCs was added as well.

In Hallmarks of cellular senescence section, SA-beta-Gal staining is one of the commonly used assays to detect cellular senescence and related with the loss of proteostasis (PMID: 36599349, PMID: 11927518).

SA-B-gal was included in markers of senescence, references added.

The urinary α-Klotho was also identified as a senescence marker which may be a useful test for clinical trials.
In addition to DPP4, there are several newly identified markers of cellular senescence including PD-L1(PMID: 36323784), uPAR(PMID: 32555459), GPNMB(PMID: 37117524), CD153(PMID: 32424156).

Other newly identified markers of senescence include PD-L1(PMID: 36323784 teh-wei wang, uPAR (PMID: 32555459 corina amor, GPNMB PMID: 37117524 masayoshi suda, and CD153 PMID: 32424156 shota yoshida. The urinary marker -Klotho was also recently identified as non-invasive way to measure cellular senescence that may be useful in clinical trials (Yi Zhu).
These markers of senescence were added.

In the paragraph of endothelial dysfunction, most of the evidence are supported by in vitro study. The paper using endothelial specific p53 knockout mice might be considered as a in vivo study (PMID: 30790589).

This study was added.

The paragraph about senolytics and ferroptosis is interesting, but it is too much focused in spite of the small amount of evidence. As the authors mentioned like "information on their role in the context of aging and senescence is deficient." in the manuscript, most of the experiments were done by in vitro or ex vivo and there is no evidence in the context of

CVDs, vascular function or endothelial cells. I wonder if this paragraph is necessary in the review tiled “Targeting Senescence in Cardiovascular Disease with Aging”.

“ in clinical settings” should be added to the last paragraph “Currently, there is not enough research on the use and treatment of senolytic therapy in cardiovascular diseases.”

“In clinical settings” was added to last paragraph.

A review should summarize literature in a critical manner. Thre is evidence that elimination of senescent pulmonary endothelial cells by ABT263 or FOXO4-DRI interventions may worsen pulmonary hemodynamics (PMID: 36515093). More studies about long-term effects or side-effects should be done.

Although elimination of senescent cells has shown promising results in cardiovascular and age-related diseases, examination of potential off-target or unanticipated side effects of senolytic therapy are also needed. Indeed, although a study examining pulmonary arterial hypertension (PAH), demonstrated high lung p16, p21, and γ-H2AX protein levels as well as increased vascular senescence and DNA damage in patients with PAH, a detrimental effect of senolytic treatment has been described in the pulmonary circulation. For example, in a mouse model of hypoxia, eliminating senescent cells via the senolytics ABT263 or FOXO4-DRI aggravated the severity of monocrotaline-induced pulmonary hypertension(PMID: 36515093). This leads to the possibility that senolytics interventions may worsen pulmonary hemodynamics and strategies targeted at the elimination of senescent cells should consider the potential impact of senescence on the pulmonary system. More studies are necessary to better elucidate pulmonary senescence and the possibility of senescence as a protective mechanism against pulmonary hypertension and progression.

“toward higher levels of ABT42, a biomarker that is inversely related to Alzheimer’s disease diagnosis. 65”
ABT42 should be typo. It is AB42. This reference 65 is preprint. The correct reference is PMID: 37679434.

Corrected.

The name of the journal should be bold in the reference 40.

Reference 40 was deleted.