nature portfolio

Peer Review File

Antagonistic stem cell fates under stress govern decisions between hair graying and melanoma

Corresponding Author: Professor Emi Nishimura

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Cell Biology.

Version 0:

Decision Letter:

Our ref: NCB-A57990-T

28th May 2025

Dear Dr. Nishimura,

I am writing on behalf of my colleague, Dr. Stylianos Lefkopoulos, who is out of the office.

Thank you for submitting your revised manuscript "Stem cell senescence-differentiation vs. bypass governs hair graying and melanoma fate" (NCB-A57990-T). It has now been evaluated together with your rebuttal to the original referees' concerns by the editorial team, and we find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Cell Biology, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines.

If the current version of your manuscript is in a PDF format, please email us a copy of the file in an editable format (Microsoft Word or LaTex)-- we can not proceed with PDFs at this stage.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Cell Biology Please do not hesitate to contact me if you have any questions.

Sincerely,

Christina Kary, PhD
Chief Editor
Nature Cell Biology
1 New York Plaza

Version 1:

Decision Letter:

Dear Emi,

I am pleased to inform you that your manuscript, "Antagonistic stem cell fates under stress govern decisions between hair graying and melanoma", has now been accepted for publication in Nature Cell Biology. Congratulations!

Thank you for sending us the final manuscript files to be processed for print and online production, and for returning the manuscript checklists and other forms. Your manuscript will now be passed to our production team who will be in contact with you if there are any questions with the production quality of supplied figures and text.

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Please feel free to contact us if you have any questions.

With kind regards,
Stelios

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Response to Reviewers

Referee #1 (Remarks to the Author):

This is a beautifully revised manuscript, and I have no further comments. I congratulate the authors on a nice study that will add significant value to the aging and cancer field.

Our response

Thank you for fully understanding our points, including the human relevance, and for recognizing the value of our revised manuscript in the fields of ageing and cancer.

Referee #2 (Remarks to the Author):

The authors have addressed my major concerns from the prior review. Importantly, they have clarified the confusion that I had regarding "depleting stress" vs. "cancer inducing stress" with new experiments and revision of the manuscript text. They also now show that dmba induces Kitl expression, which makes an interesting mechanistic advance in the field. Overall, this manuscript is an incredible advance for the fields of stem cell and cancer biology and will be well cited for these fields.

Our response

Thank you for recognizing the advances in our manuscript and its potential impact on the fields of stem cell and cancer biology. We sincerely appreciate your thoughtful review and valuable feedback.

Referee #3 (Remarks to the Author):

Comment #1:

This is a revised manuscript, that has addressed some of the points raised in the first review, but that continues to maintain that they have identified a "tissue phenotypic trade-off between ageing and cancer." I don't see this in the work, and continue to be confused the fact that aging is the biggest risk factor for melanoma in skin, and most people who are older have grey hair. One interpretation of this work is that if we didn't have grey hair, we would get more scalp cancers ? This seems to be what they are showing in Figure 5b (: new Fig. 6b).

Our response

Thank you very much for your thoughtful comments and for raising an important point. We understand your concern and appreciate the opportunity to further clarify our interpretation. While we addressed this point in our earlier response, we realize that our previous explanation may not have been fully clear.

We fully acknowledge that both hair graying and melanoma incidence increase with age. However, our study focuses on stress-induced antagonistic mechanisms within individual McSCs that govern stem cell fate decisions. A key concept is that the fate of all McSCs is not synchronized; rather, each McSC independently makes its own fate decision. Thus, we do not suggest that skin fate follows a simple trade-off model at the tissue level, but rather that the cumulative outcome of individual cell fates shapes tissue-level phenomena.

In mouse hair follicles, synchronized hair cycle, controlled environmental stressors, and genetic uniformity allow the fate of individual McSCs to more directly influence tissue outcomes. In our experiments, hair graying associated with McSC depletion suppressed melanoma formation (new Fig. 6e). However, when some McSCs remain after IR irradiation and subsequently acquire melanoma-inducing mutations, both hair graying and melanoma can coexist (as shown below (left) and in new Fig. 6e). This context-dependent phenomenon is particularly relevant to human skin, where stress exposure and mutational landscapes are far more heterogeneous. Our conclusions are drawn by shifting the perspective from a population-level analysis of stem cells to a single-cell fate tracing, which captures the diverse outcomes of stem cells under genotoxic stress.

To avoid misinterpretation, we have now carefully removed the phrase “phenotypic trade-off between ageing and cancer” from the manuscript. We have also revised the text and updated the schematic in new Fig. 6g (: previous Fig. 5) to more explicitly convey our findings (as shown below (right) and in new Fig. 6g).

We truly appreciate your insights and your engagement with the conceptual implications of our work. We hope the revised explanation and updated figures help clarify our perspective.

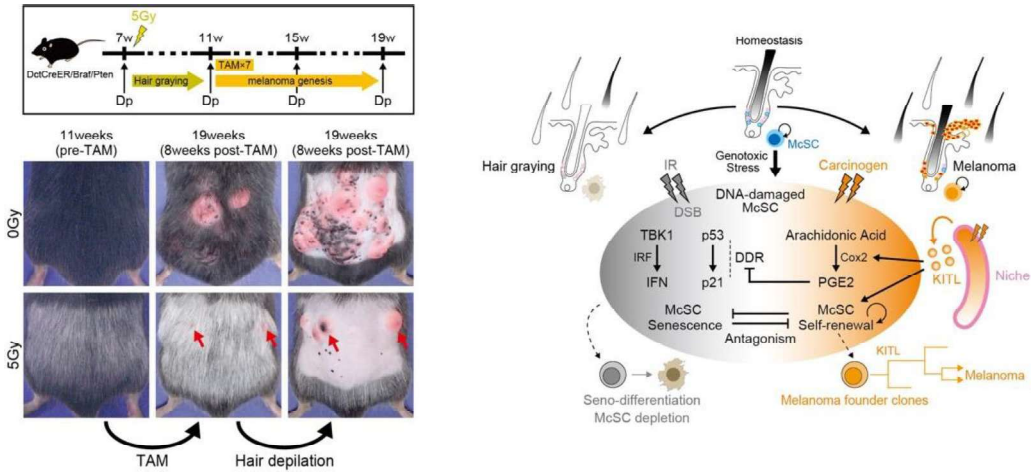

Figure for Reviewer #3

Comment #2:

Probably the significance of this would be better realized if they would speak directly to what they have found, which again, I find this the most interesting aspect of the work: that different DNA damaging agents cause differential effects on melanocyte stem cells, and that DNA damage niche-derived KITL is physiologically essential for the maintenance of McSCs and can resuce IR/DSBs induced genotoxic checkpoints.

Our response

Thank you very much for highlighting what you identified as the most interesting aspect of our work. We fully agree that the finding that distinct types of DNA damage elicit differential responses in McSCs—and that these responses are modulated by niche-derived KITL—is a key insight of the study. We truly appreciate your recognition of its significance and your thoughtful articulation of its implications.

We have taken care in the revised manuscript to make this mechanistic point more explicit, and we would be delighted to discuss this further with you in the future.

Comment #3:

It is unhelpful to categorise the DNA damaging agents as genotoxic versus carcinogenic, as we know IR is both. It is also unhelpful and confusing to use the "seno-differentiation" term, as they really don't do many experiments on actual aged animals

(although they do a few). Finally, I maintain that the relevance for melanoma is not clear, given they are look at hair follicle stem cells in a mouse (which would be similar to our hair), that lack the epidermal melanocytes in normal human skin. The experiments here don't represent melanoma that is seen in human aging, so is not really about an aging-cancer axis.

Our response

Thank you very much for your thoughtful comments and for raising these important points. We understand that some of the terminology used in the earlier version may have led to confusion, and we appreciate the opportunity to clarify our rationale. As noted by Reviewer #2 and Reviewer #4, similar concerns were previously raised, and we have carefully revised the manuscript to address them. In the revised version, we have distinguished between 'stem cell-depleting stress' and 'carcinogenic stress', categorizing IR/DSB as 'stem cell-depleting' based on our experimental observations. This terminology is consistent with the clinical use of IR in cancer therapy and its well-known effects on tissue atrophy.

Regarding the term 'senescent cell', it is now well recognized that senescent cells can be induced by a variety of genotoxic stresses and are not limited to aged tissues. The prefix 'seno', as used in terms like 'senolytic' and 'senotherapy', has gained broad acceptance to denote stress-induced cellular senescence. In addition, we have demonstrated that 'seno-differentiation' is not lineage-restricted but may represent a generalizable process, as our findings in keratinocytes also support this (Kato et al., 2021, Dev. Cell). In fact, we have used the term 'seno-differentiation' of McSCs at multiple conferences on ageing and cancer, where it has been well received.

We appreciate your concern regarding the model system. It is true that the mouse epidermis lacks melanocytes in hair-bearing regions where KITL expression level is low. However, when our mouse model is combined with UVB irradiation (new Fig. 6c), it develops a dermoscopic features that closely resemble human lentigo maligna melanoma, including asymmetric pigmented follicular openings - a well-established dermoscopic term - which is classified as a high CSD-melanoma. Based on this, we believe our model provides relevant mechanistic insights into human melanoma, particularly within a genotoxic stress context.

In summary, we see one of the key contributions of our work as the identification of stem cell fate bifurcation—exhaustion versus expansion—at the clonal level of McSCs, along with the distinct pathways that respectively underlie pigmented tissue ageing and cancer development. We hope that these clarifications and revisions make our perspective clearer, and we are sincerely grateful for your thoughtful insights, which have strengthened the manuscript.

Comment #4:

The authors don't account for newer work in human melanocyte stem cells that show reversibility https://elifesciences.org/articles/67437

Our response

We appreciate the reference provided by Reviewer #3. The cited study offers valuable insights into melanocyte stem cell reversibility in human hair. However, it primarily analyzes pigment production in the hair bulb during the hair cycle and does not specifically address McSC fate, which is primarily determined during early anagen.

While a subset of intermediate melanocytes (McSCs) can dedifferentiate, this occurs much later during the hair cycle without influencing the color of the existing hair but potentially contributing to the pigmentation of newly grown hair.

It is well established that within a single hair cycle, fluctuations in hair color are governed by the differentiation status, number, and activity of mature melanocytes in the hair matrix, rather than on McSCs. McSCs contribute to generating their progeny, which migrate into the hair matrix only during early anagen. Therefore, any changes in hair color occurring within a single hair cycle are independent of McSC activity. This mechanism appears to be conserved across species, including both mice and humans. We sincerely appreciate your thoughtful feedback and the opportunity to further clarify this important point in our manuscript.

Comment #5:

Minor point - it looks like BRAF expression on its own is causing mild hair greying (Figure 5b (: new Fig. 6b)).

Our response

Thank you for your careful observation. We agree that in some cases, TyrCreER/Braf^{CA} mice exhibit slight hair graying. However, as you noted, this effect is relatively mild and not comparable to the robust hair graying observed following IR exposure. More importantly, the significant hair graying observed in WT mice under IR irradiation is markedly suppressed in TyrCreER/Braf^{CA} mice, supporting the conclusion that Braf signaling mitigates McSC depletion and prevents IR-induced hair graying.

Referee #4 (Remarks to the Author):

The manuscript has been revised in a very thoughtful manner. It is now easy to follow and engaging to read, I really enjoyed reading the new version. I have some minor suggestions that could further improve clarity.

While the title is catchy, to me it does not convey a clear message. The phrase “and its bypass” in connection with “the key” is particularly vague. Reconsidering the title could help to present a more clear and thus more impactful message.

Figure (4b) The image would benefit from an outline of the major skin structures (IFE, infundibulum, SG, bulge, etc.); the current figure item does not add much and is better seen in Fig S7A. (4h) Consider specifying "early anagen" for consistency in labeling.

Our response

Thank you for your positive feedback and for finding our manuscript engaging and enjoyable to read.

In response to your suggestion, we have modified the title of our manuscript to: “Antagonistic stem cell fates under stress govern decisions between hair graying and melanoma”. We believe this revision enhances clarity and impact. In addition, as you recommended, we have revised Figure 5 (previous Figure 4) by highlighting the major clusters (new Fig.5c) and specifying ‘early anagen’ for consistency in labeling.