Enhanced mTORC1 Pathway Leads to Melanocyte Stem Cell Aging Through DNA Damage

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Abstract

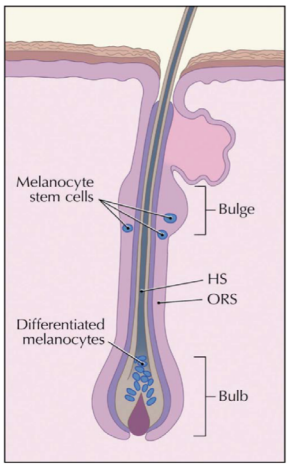
The aging of skin stem cells is always attractive and highly relevant to our daily lives. Hair graying, mainly caused by melanocyte stem cell (MeSC) aging, is a severe problem for many people. Previous studies have pointed out that DNA damage is a significant reason for MeSC aging, and the enhanced mTORC1 pathway leads to DNA damage. Here, experiments in vivo and in vitro were designed to verify that enhanced mTORC1 pathway leads to melanocyte stem cell aging through the cause of DNA damage. The research could have the significance of discovering possible reasons for hair graying and providing some new ideas in treating this abnormal divergence and other diseases relevant to cell aging.

Keywords: mTORC1, melanocyte stem cells, cell aging, DNA damage, hair graying

1. Introduction

1.1 *Previous Studies*

Hair graying, caused by progressive loss of hair melanin, is a serious symbol of human aging, which annoys lots of people for their appearances. In normal aging, hair graying occurs at 34±9.6 years of age in Caucasians, and 43.9±10.3 years in African Americans, when their hair follicles pass through certain life cycles (normally 7-15 complete cycles) [1]. Hair graying happens mainly because the rate of replacing melanocytes cannot match the rate melanocyte is damaged and homeostasis of melanocyte cannot be maintained (see Figure 1) [2].



Note: MeSC exist in the bulge area of hairs and differentiate into melanocytes, which produce the pigment melanin that causes dark hairs.

Figure 1. Melanocyte Stem Cells and Hair Graying [2].

There are some previous studies concerning the reasons and biomarkers of melanocyte stem cells aging. The depigmentation of newly grown hairs, is led by MeSC aging (Ectopic Differentiation of MeSCs), which causes the loss of melanocytes that produce melanin. Therefore, hair graying could be one of the most obvious aging phenotypes of MeSC. On the other hand, the ectopically pigmented melanocytes (EPMs), which are in types of dendritic, often occur in aged melanocyte stem cells, suggesting the differentiation of them. In that case, the appearance of dendritic morphology cells could also be a biomarker of MeSC aging [3].

Previous studies also point out that MeSC aging is most likely to be caused by DNA damage. The use of excessive genotoxic stress (can cause DNA damage) results in the differentiation of stem cells in the niche by the chronological fate analysis of them. This significant change has soon led to stem cell depletion and hair graying. The two well-studied diseases, Werner's syndrome and Ataxia-telangiectasia (AT), are caused by abnormalities at the genetic level, for instance the break of DNA double-stranded structure. These also represent that the variation of DNAs is an essential reason for skin cell diseases. Researches also point out some "caretaker genes", which are related to stem cells aging. The deficiency of ATM gene, a kind of "caretaker genes" accelerates the hair graying of mice. [4].

The mammalian target of rapamycin (mTOR)is a widely studied signal pathway in mammal cells. It forms to different compounds of mTORC1 and mTORC2 through interactions with proteins. Researches shows that mTORC1 pathway highly associates with DNA damage in mammal cells. Two special proteins, O-6-

methylguanine-DNA methyltransferase (MGMT) and N-myc downstream-regulated gene 1 (NDRG1) that play roles in DNA repair, are negatively regulated by mTORC1 in senescent mice and cells. Therefore, the enhanced mTORC1 pathway may lead to DNA damage, and can be detected by the two proteins [5].

Methods in activating mTOR pathway in mammal cells are also discussed widely. Two signal pathways, Akt and ERK, promote mTORC1 signaling through phosphorylation of a GTPase activator protein (GAP), referred to as tuberous sclerosis complex 2 (TSC2), that acts as an upstream inhibitor of mTORC1. The ERK pathway can be promoted by LPA, and Akt pathway can be promoted by insulin. The two signaling pathways are in a collaborative relationship in the promote of mTORC1 pathway [6]. So mTORC1 pathway can be activated or promoted under LPA and insulin treatment. Triamcinolone Acetonide, a synthetic glucocorticoid, which prevent the knock down of lysosomal that inhibit mTOR pathway in vivo, could also be a promotor of mTORC1(see Figure 2) [7]. And rapamycin, a macrolide produced by the bacterium Streptomyces hygroscopicus and first discovered in soil samples from Easter island, is a well-known medicine in inhibiting mTORC1 pathway (see Figure 3) [8].

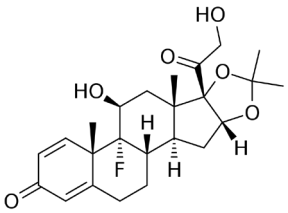


Figure 2. Triamcinolone Acetonide.

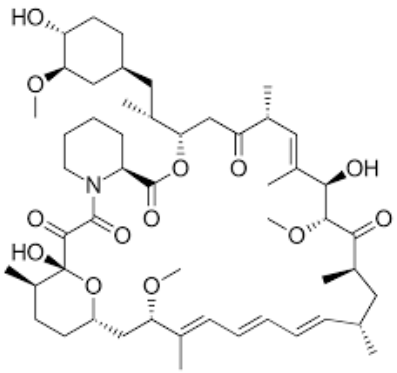


Figure 3. Rapamycin.

1.2 *Hypothesis*

Due to previous studies that the DNA damage can be a causing reason for MeSC aging, and enhanced mTORC1 could lead to DNA damage in cells. The above aspects and conclusions could be associated together. Similar researches have also confirmed that mTORC1 pathway leads to the aging of hair follicle stem cells (HFSC) [9]. Therefore, the research would like to come up with the hypothesis that enhanced mTORC1 signal is one reason that might lead to MeSC aging through DNA damage. Knowing that rapamycin is a inhibitor of mTORC1, and the DNA damage caused by enhanced mTORC1 can be detected by NDRG1 & MGMT proteins. If the hypothesis stands, the MeSC will become aging (hair graying) when mTORC1 pathway is promoted. And the expression of NDRG1 & MGMT proteins will be lower in experimental group (mTORC1 is promoted) than control groups (promoted mTORC1 is then inhibited). If the hypothesis isn't correct. The hairs will not turn gray under treatments of activation or promotion. Or the hairs turn gray, but the expression level of the two proteins shows no differences with the control group.

1.3 *Research Goals*

As a common symptom of aging, hair greying seems to trouble more and more people nowadays. The goals of this research is to discover the molecular mechanisms that lead to melanocyte stem cells aging and the hair greying, which brings a few ideas for hair greying treatments. The study would also like to find out more about the synergistic relationship between mTORC1, DNA damage, and skin stem cell aging to further explore this physiological pathway in human body.

2. Methods and Materials

2.1 *Animals and mTORC1 promotor*

There are three stages of experiment of this research. The first set of experiment should be done in vivo. It uses triamcinolone acetonide as an mTORC1 promoter (triamcinolone acetonide bolus injections: TAA suspension, 40 mg/mL, Janssen Pharmaceutical Ltd. XI'an China diluted in 0.9% sterile saline. In order to enhance accuracy, the study has designed two approaches for this experiment. First, we set 36 Dct-lacZ (see Figure 4, extensively used to study melanocyte biology) transgenic mice, they would be divided into three groups, group 1, group 2, and group 3. Group 1 and group 2 receive injection of triamcinolone acetonide, group 3 receives equal amounts of stroke-physiological saline. After eight weeks, compare group 1 and group 2's hair color with group 3 (see Figure 5). Then, group 1 would receive rapamycin (5mg/kg, Janssen Pharmaceutical Ltd. XI'an China) (enterocoelia).

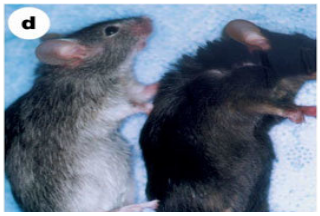


Figure 4. Dct-lacZ mice [10].

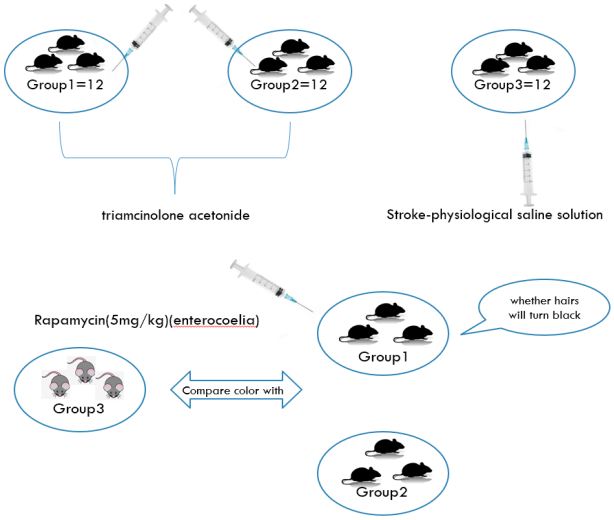
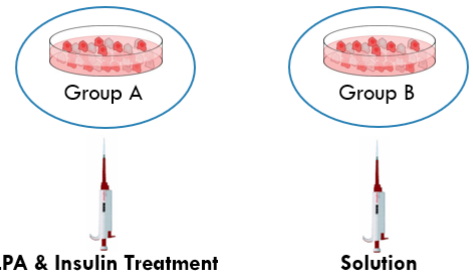


Figure 5. Group1/Group2 receive injection of triamcinolone acetonide(enterocoelia)(40g/L/d) for 8 weeks, Group3 receive equal amount of Stroke-physiological saline solution (Owner-draw).

Compare hair color between Group1/Group2 and G3. Then Group 1 receive rapamycin and test whether hairs will turn black. An alternative method for this experiment is to inject rapamycin to group 1 at the same time triamcinolone acetonide is injected, the rest treatments do not change. And the color between group 1 and group 2, as well as group 2 and group 3 should have significant differences.

2.2 *Cell Culture*

To better verify the hypothesis and avoid possible weaknesses of the in vivo experiment. A set of in vitro experiment through cell culture is also designed, following Jeremiah N. Winter,etc's methods [6]. In this experiment, two groups of melanocyte stem cells are cultivated in Dulbecco's modified Eagle's medium (DMEM medium) (suitable for stem cell growing). Group A, the experimental group receive treatment of 2 hours, 22 μM LPA (Sigma-Aldrich. USA), and 10 nM insulin (Novo Nordisk. USA) (see Figure 6). The control group, group B receive equal amount of solution. Then the occurrence of ectopically pigmented melanocytes (EPMs) is detected in the two groups through electron microscopy (JEOL).



Note: The group A receive LPA and Insulin treatment for 2 hours, while Group B receive equal amount of solution with Group A.

Figure 6. Treatments of the in vivo groups (Owner-draw).

2.3 *Protein detection and Real-time qPCR*

To verify that it is DNA damage caused by enhanced mTORC1 that lead to MeSC aging. The expression levels of NDRG1 & MGMT proteins should be measured through real-time qPCR. The experiment should be done in vitro, thus the mice of in the first experiment should be killed under ethical rules. Then tissues are collected from each mouse, froze with liquid nitrogen, and store at −70 °C for western blots. The cultivated cells in the second experiment are scraped into 1× Laemmli buffer for western blots. The probes for PCR are listed in Table 1.

Table 1. Probes of RT-qPCR to measure protein expression levels [5].

Probe		Sequence
MGMT	Forward	AAACACTGACCCCACAGAGG
	Reverse	AACACAGGGTGATGGAGAGC
NDRG1	Forward	CGAGAGCTACATGACGTGGA
	Reverse	AAGAGGGGGTTGTAGCAGGT

3. Results:

3.1 *Hair Color Variations in Mice*

If the hair color of group 1, group 2 is grayer than group 3(p equal to or smaller than 0.05 is significant), and after the injection of rapamycin, the hairs of group 1 turn black, then mTORC1 is a cause for MSC aging. If hairs of group 1 and group 2 turn gray but didn't turn black after rapamycin, then the triamcinolone acetonide leads to MeSC aging, but through other mechanisms. If hairs of group 1 and group 2 didn't turn gray, then mTORC1 is not a cause of MeSC aging. Under the situation of the alternative approach, if the group 1 has significant hair color difference with group 2, as well as group 2 and group 3. This also leads to the conclusion that enhanced mTORC1 signal pathway leads to MeSC aging.

3.2 *EPM cell occurrence with enhance mTORC1 signal*

If cells in group A occur ectopically pigmented melanocytes (see Figure 7), and cells in group B do not have the same phenomenon. This comes with the same conclusion that mTORC1 is a cause for MeSC aging.

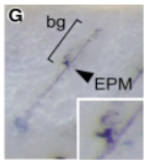
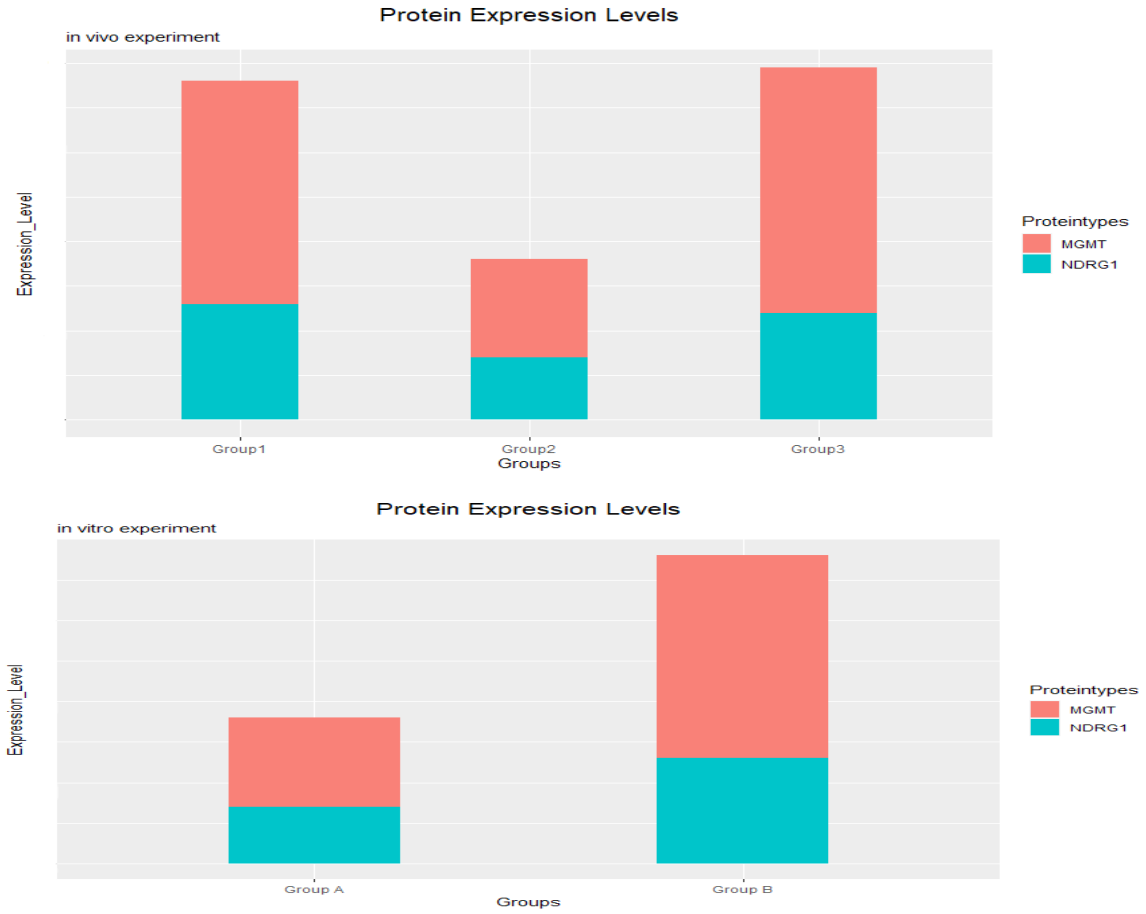


Figure 7. Shapes of ectopically pigmented melanocytes(Owner-draw).

3.3 *Expression Levels of DNA Damage-relevant Proteins*

If expression levels of MGMT and NDRG1 are lower in Group 2 than Group 1&3, and lower in group A than Group B(see Figure 8). The conclusion is that the aging of MeSCs lead by mTORC1 is through DNA damage. If the expression levels of MGMT and NDRG1 shows no remarkable difference between each group. It might be considered that mTORC1 causes MeSCs aging, but through other mechanisms other than DNA damage. Through this experiment, the research can test the conclusion that the cause for MeSC aging under promoted mTORC1 is DNA damage.



Note: Expression of MGMT (red bar) and NDRG1 (green bar) proteins are lower in Group 2 than Group 1&3, and lower in group A than Group B.

Figure 8. Expected Protein Expression Levels (Owner-draw).

4. Discussion

4.1 *New Associations with mTOR, hair graying, stem cells, and DNA damage*

As the increasing pressure in people's lives, gray hair and hair loss seem to be becoming an increasingly serious problem. The main purpose of the research is to link the known principles and conclusions together with two separate experiments and one experiment together. It may have significance for people to prevent hair graying from aspects of daily foods, medicine intakes, exercise, and other life matters related to mTOR pathway in body.

The research points out a way to study one of the causing reasons for skin stem cells aging. Stem cells have the ability of self-differentiation, which is essential for the life functions and metabolism of the body. On the other hand, aging of stem cells also relevant to other diseases such as cancers [11-13]. We look forward to more associations with aging and other human diseases.

4.2 *Multiple experimental methods in verifying the hypothesis*

In the first part of the experiment, after the mTORC1 pathway is promoted or activated, it uses rapamycin to inhibit the signal and see whether the gray hairs can turn black again. However, this could be improper because the aged melanocyte stem cells may not turn back to the previous niche even when the mTORC1 pathway is inhibited again. Or the gray hair may not turn black again due to certain reasons. Therefore, the alternative approach that inject with triamcinolone acetonide and rapamycin at the same time might solve this issue. The mTORC1 pathway is inhibited once promoted may cause less irreversible cell damages. In addition, the injection volume under this approach should be considered.

4.3 *Other possible flaws or inaccuracies*

Other weaknesses of this research including the biomarkers are not complete. The color of hairs or the occurrence of EPM cells are likely to be inaccurate, more quantitative traits for MeSC aging measurement could be discovered. The mTORC1 promoters and inhibitor rapamycin may also have functions in controlling other metabolism pathways, which need more studies to remove possible inaccuracies.

5. Conclusions

The research studies a new concept of hair greying. Melanocyte stem cell aging is one of the important causes of white hair. Here we explore one possible factor leading to hair graying—the mTOR pathway leads to DNA damage, and lead to melanocyte stem cells aging and hair graying. As a topic highly relevant to medical researches, we also discovered more spaces of mTOR pathway researches [12].

Future studies may focus on the other explanations of the results in this research. For instance, the reactions with mTORC2 and other metabolism pathways and the promotor and inhibitors we use here. Other biomarkers as to measure the aging of skin stem cells could also be discovered and contribute to this issue.

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