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Autophagy as a Melanocytic Self-Defense Mechanism

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Abstract

Defects in autophagy have implications for melanocyte survival and manifestations of skin pigmentary disorders. Zhang *et al.* (2015) show that mouse melanocytes lacking the autophagy protein Atg7 undergo premature senescence *in vitro* and accumulate products of oxidative damage, despite activation of the redox response. Interestingly, contrary to previous findings, the melanocyte-specific deficiency in autophagy did not cause major defects in melanosome biogenesis, nor did it produce visually striking changes in mouse coat color.

In this study, to test the role of autophagy in melanocytes, Zhang *et al.* (2015) deleted Atg7 specifically in melanocytes using floxed- Atg7 and Tyr::Cre mice. Autophagy, often thought to be activated in response to cellular starvation, is now considered an important cell biological pathway involved in regulation of wide ranging biological processes, including tissue homeostasis. Accordingly, defects in autophagy are being increasingly recognized in many pathological conditions. Possible involvement of autophagy in a melanocytic disorder was suggested >35 years ago (Hirobe and Ervu, 1978). Detailed understanding of vesicular pathways and the molecular factors involved in the biogenesis of autophagosomes (Lamb *et al.*, 2013) and melanosomes (reviewed in Sitaram and Marks, 2012) spurred recent investigation into the relationship between autophagy and melanosome biogenesis, pigmentation, and pigmenary disorders (Smith *et al.*, 2005; Ganesan *et al.*, 2008; Kalie *et al.*, 2013).

Role of autophagy in melanocytes: a genetic approach

Zhang *et al.* (2015) used a biochemical parameter—conversion of LC3-I to LC-3II—to monitor autophagy in wild-type and Atg7-deficient melanocytes. The investigators also monitored accumulation of the p62/sequestosome 1(SQSTM1), a ubiquitin, and LC3-binding protein that is degraded upon activation of autophagy, as an indication of defective autophagy. The major findings of this study are that constitutive autophagic activity has a role in preventing premature senescence and oxidative damage in melanocytes, but with little effect on melanin pigmentation.

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CONFLICT OF INTEREST

The author states no conflict of interest.

Effect of autophagy on pigmentation: conspicuous by absence

Disruption of mouse melanocyte cellular function often leads to readily visible changes in coat color due to either altered numbers of melanocytes or alterations in the amount and distribution of melanin in melanocytes and keratinocytes. Therefore, Zhang *et al*. (2015) characterized coat color defects and melanin content in dorsal coat hair, cultured melanocytes, and keratinocytes. On the basis of these observations, they concluded that Atg7-dependent cell biological processes are dispensable for melanosome biogenesis, melanin synthesis, and its export to keratinocytes, but that it may be required for full pigmentation of hair and epidermis. This conclusion was based on the observation that, in Atg-null mice, pigmentation of tail skin is decreased at the age of 9 months and that there is only a small, but significant, decrease in melanin content in dorsal hair. However, the visual effect on coat color is not striking.

Much of what we have learned about the consequence of Atg7 deficiency in melanocytes is based on their behavior *in vitro*. First, although the melanin content of coat hair in *Atg7f/f*-Tyr::Cre mice was reduced, no differences were noted in the amount of intracellular melanin in wild-type and Atg-null melanocytes that were cultured from neonatal skin.

In a previous study, Ganesan *et al.* showed that heterozygous deletion of the autophagy protein *Beclin 1* in mice results in lighter coat color. However, the Beclin1 heterozygous-null mice did not exhibit uniformly lighter coat color because of the coexistence of both normal and hypopigmented hair follicles. On the basis of immunohistochemical staining for a melanoblast marker protein, Ganesan *et al.* concluded that the lighter coat color in mice with Beclin 1 haplosufficiency is not due to decreased melanocyte survival but due to decreased melanosome numbers or melanin content within hair follicles. Although Zhang *et al.* (2015) acknowledge the discrepancy between their observations and those of Ganesan *et al.* on coat color, they did not present possible reasons for this discrepancy.

Autophagy and melanocyte proliferation, and senescence *in vitro* and *in vivo*: an enigma

In this study, Zhang *et al.* (2015) showed that Atg7 deficiency resulted in decreased proliferation and premature senescence, as indicated by higher expression of p16Ink4a. They also found that, in Atg7-deficient mice, melanocyte density in the tail epidermis showed a tendency to be lower compared with that in wild-type mice, but the difference was not statistically significant. The significance of the reduced proliferation and increased senescence *in vitro* to melanocyte density in the skin is not clear because these investigators did not evaluate the senescence status of the skin-resident melanocytes *in vivo.* Staining epidermal sheets for senescence-associated beta-galactosidase and/or p16Ink4a could have proven valuable in establishing the significance of these *in vitro* findings. Additional studies are clearly warranted to understand the role of autophagy in determining the density and nonrandom distribution of melanocytes in the skin.

Regulation of Nrf2, redox enzymes, and oxidative stress by autophagy: potential connection to vitiligo

The finding that ubiquitinated p62/SQSTM1 accumulates in Atg7-deficient melanocytes led Zhang *et al.* (2015) to assess the relationship between autophagy and cellular redox homeostasis. Interestingly, transcription of *p62/Sqstm1* is upregulated by the redox-sensitive nuclear factor (erythroid-derived 2)–like 2 (Nrf2), and Komatsu et al. have shown previously that the autophagy substrate p62 activates Nrf2 through inactivation of Keap1. Thus, positive feedback appears to exist between redox signaling and autophagy. Such a relationship has been proposed and studied extensively in other systems (Filomeni *et al.*, 2014). Interestingly, in Atg7-deficient melanocytes, Zhang *et al.* (2015) found that, although expression levels of Nrf2 did not change, there was induction of several canonical Nrf2 target genes such as *NADPH quinone oxidoreductase (Nqo1)* and *glutathione S-transferase mu 1* (*Gstm1*), measurable at both mRNA and protein levels. Surprisingly, *hemeoxygenase 1* (*Hmox1*), which is also an Nrf2 target gene, was not induced in Atg7-deficient melanocytes. Zhang *et al.* (2015) suggest a role for cell type–specific regulation of *Hmox1*. This warrants further investigation. Paradoxically, activation of Nrf2 and induction of Nrf2-responsive redox enzymes increased the oxidative stress in Atg7-deficient melanocytes as indicated by increased staining with a (ROS) reactive oxygen species-sensitive probe and increased lipid peroxidation. To explain this paradox, Zhang *et al.* (2015) hypothesize that a feedback relationship exists between autophagy and lipid oxidation; that is, although deficiency in autophagy leads to increased lipid peroxidation, autophagy in turn serves to sequester or dispose of the oxidized lipids.

These findings are highly relevant in understanding human pigmentary disorders. A defective response to oxidative damage and escape from oncogene-induced senescence are critical attributes of vitiligo and melanoma—two melanocytic disorders at the opposite ends of a spectrum of premature death and uncontrolled proliferation. On the basis of the similarity between Atg7-deficient and vitiligo phenotypes, specifically with respect to the activation of Nrf2 regulated genes, oxidative stress, and premature senescence, Zhang *et al.* (2015) propose a model in which autophagy-deficient melanocytes and vitiligo melanocytes share defective cellular redox regulation, increased membrane lipid oxidation, and premature senescence. However, the role for Atg7 and autophagy in premature melanocyte death in vitiligo remains to be established. Similarly, these studies do not yet provide clues to resolving the ongoing debate (tumorigenic vs. tumor suppressor) on the role of autophagy in melanoma.

Autophagy and melanocytes: unanswered questions

This study by Zhang *et al.* (2015) raises several questions. First, why does Beclin 1 haploinsufficiency, but not loss of Atg7, produce a coat color defect? One possible explanation is that, whereas Zhang *et al.* (2015) deleted Atg7 specifically in melanocytes, Ganesan *et al.* studied mice with one copy of the Beclin1 gene deleted in all cells. This raises the possibility of a melanocyte nonautonomous role for autophagy in regulating coat color. Alternatively, as autophagy has been shown to occur independently of Atg7 (Nishida *et al.*, 2009), the function of this protein may also be dispensable during melanogenesis. A

direct comparison of mice in this study with mice with melanocyte-specific deletion of *Beclin1* may resolve this issue. Second, what is the role of autophagy in melanocyte senescence? Although Zhang *et al.* (2015) noted premature senescence of Atg7-deficient melanocytes *in vitro*, there was no evidence of increased senescence of melanocytes *in vivo*, neither in the Atg7-deficient nor in haploinsufficient Beclin1 mice. Zhang *et al.* (2015) invoke accelerated proliferation *in vitro* as a possible reason for senescence, and they propose that such a state may not be reached *in vivo*. However, this remains to be proven. A possible way to study this question would be to determine whether Atg7-deficient melanocytes exhibit heightened sensitivity to oncogene-induced senescence compared with their wild-type counterparts. This will be relevant in understanding the role of autophagy in the susceptibility to melanoma development. Third, what role does autophagy have in melanocyte response to ultraviolet B (UVB) radiation exposure? Although Zhang *et al.* (2015) studied the effect of exposure to UVB radiation on senescence and redox homeostasis, investigation of the differences in survival between wild-type and Atg7-deficient melanocytes after exposure *in vivo* in mouse skin could provide insight into the significance of these studies. Additional studies are clearly warranted to establish an unambiguous role for autophagy in melanocyte biology and skin pigmentation.

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Clinical Implications

- The investigators studied the consequences of loss of *Atg7* gene function specifically in mouse melanocytes using a genetic approach.
- By demonstrating that Atg7 and autophagy are dispensable during melanogenesis *in vitro* and *in vivo*, this study contradicts earlier studies that had suggested a role for autophagy in melanogenesis.
- Importantly, this study uncovers a role for Atg7 in regulating the oxidative stress response and senescence of melanocytes, with implications for a similar role in melanocytic disorders such as vitiligo, where defective reactive oxygen species responses and premature senescence are found.
- Methods to evaluate and monitor autophagic activity in epidermal melanocytes may be of value for the early diagnosis and treatment of vitiligo.