

## Article Addendum

# Autophagy and aging

## New lessons from progeroid mice

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It is widely-assumed that the autophagic activity of living cells decreases with age and probably contributes to the accumulation of damaged macromolecules and organelles during aging.<sup>1-3</sup> Over the last few years, the study of segmental progeroid syndromes in which certain aspects of aging are manifested precociously or in exacerbated form, has increased our knowledge of the molecular basis of aging. We have recently reported the unexpected finding that distinct progeroid murine models exhibit an extensive basal activation of autophagy instead of the characteristic decline in this process occurring during normal aging.<sup>4</sup> Further studies on *Zmpste24*-null progeroid mice, which are a reliable model of human Hutchinson-Gilford progeria, have revealed that the observed autophagic increase is associated with a series of metabolic alterations resembling those occurring under calorie restriction or in other situations reported to prolong lifespan.<sup>4</sup> Here, we analyze these unexpected findings and discuss their possible implications for the development of premature aging.

Although the precise molecular determinants of aging are still very far from being completely understood, our knowledge of the molecular basis of this complex process has improved considerably, in part due to the study of segmental progeroid syndromes. These syndromes are dramatic diseases in which certain features of human aging are prematurely developed.<sup>5</sup> Progeroid syndromes can be classified into two major groups attending to their underlying molecular defects.<sup>6</sup> The first group comprises disorders in which alterations in genome-stability maintenance mechanisms lead to the development of premature aging, whereas the second group includes those syndromes caused by defects in the nuclear envelope architecture.<sup>7-9</sup> In the last few years, the study of animal models of accelerated aging has yielded interesting results which have contributed to extend our knowledge of the molecular basis of normal aging.<sup>10,11</sup> However,

no studies about autophagic activity in progeria patients or animal models showing accelerated aging had been previously reported.

On this basis, together with the increasing connections between autophagy and aging, we decided to check the basal autophagic activity in progeroid murine models caused by either defects in DNA repair machinery or alterations in the nuclear envelope structure. Surprisingly, our analyses have revealed that progeroid mice fed *ad libitum* present a clear increase in their tissue autophagic activity as compared with the corresponding controls, independently of the molecular alterations underlying their phenotype.<sup>4</sup> This unexpected finding prompted us to study the molecular determinants of this autophagy increase. For this purpose, we focused our analysis on mice deficient in *Zmpste24* metalloproteinase (Fig. 1), which show accelerated aging and are a model of human Hutchinson-Gilford progeria.<sup>9</sup> These mice present major alterations in nuclear structure due to a defect in the processing of lamin A, an essential constituent of the nuclear envelope.<sup>12</sup> We found that the observed autophagy increase is associated with mTOR inhibition and the upregulation of LKB1-AMPK axis activity (Fig. 1). In addition, these alterations were linked to significant changes in biochemical parameters, such as reduced levels of blood glucose, insulin and leptin, together with an increase in plasma adiponectin levels.<sup>4</sup> These alterations could explain the elevated LKB1-AMPK activity observed in progeroid mice as well as the reported mTOR inhibition and autophagy activation. In fact, a decrease in blood glucose levels or an increase of circulating adiponectin lead to an *in vivo* induction of AMPK activity,<sup>13</sup> which in turn inhibits mTOR activity.<sup>14</sup> Since all these alterations in blood parameters point to a deregulation of glucose and lipid homeostasis, we checked the transcriptional levels of key genes for these processes in *Zmpste24*<sup>-/-</sup> mice livers, as this tissue is a major modulator of glucose and lipid homeostasis *in vivo*. Our analyses revealed the existence of a complex metabolic shift in glucose and lipid metabolism, as assessed by the finding that key genes involved in gluconeogenesis, glycogen accumulation, fatty acid synthesis, and  $\beta$ -oxidation were clearly upregulated in mutant mice.<sup>4</sup> These metabolic alterations were linked to a substantial increase in the levels of hepatic glycogen and also to the presence of liver steatosis (retention of lipids in cells), confirming the occurrence of a profound metabolic shift in glucose and lipid homeostasis, which likely underlies the observed basal autophagy increase, mTOR inhibition and LKB1-AMPK axis upregulation in *Zmpste24*<sup>-/-</sup> mice.

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It is remarkable that the majority of the detected alterations in these progeroid mice are associated with longer lifespan rather than with the shortened longevity characteristic of these progeroid animals. In fact, autophagic activity is essential for dauer development and lifespan extension in *Caenorhabditis elegans*<sup>15</sup> and a downregulation of TOR-signaling extends lifespan in both yeast and nematodes.<sup>16,17</sup> Similarly, AMPK overexpression promotes longevity in *C. elegans*<sup>18</sup> and the metabolic alterations found in progeroid mice, as hypoinsulinemia and hypoglycaemia, increase lifespan in diverse model organisms.<sup>19,20</sup> In addition, many of the transcriptional alterations observed in key genes for glucose and lipid metabolism regulation resemble those observed in animals subjected to calorie restriction.<sup>21,22</sup> In this regard, very recent studies show that progeroid mice with defects in different DNA repair genes also exhibit an adaptive metabolic response characterized by an upregulation of gluconeogenic and  $\beta$ -oxidative pathways as well as by alterations in the glucose/insulin pathway, which resemble in many aspects the changes observed in *Zmpste24*-null mice.<sup>23-25</sup> Our novel observation that autophagy is also markedly induced in these progeroid models suggests that activation of this pathway might be part of a general metabolic shift occurring in different progeroid syndromes.

Taken together, all these observations suggest that the different molecular defects leading to the development of premature aging trigger a complex and conserved metabolic response including many features tightly associated with lifespan extension. At first sight, this fact seems intriguing. However, most of these features point towards a reduction in the metabolic activity of the organism, which inevitably leads to a reduction in cell division rate. This appears to be an adequate strategy to reduce the accumulation of cellular damage, as it has been reported that cell division drastically increases the rates of abnormal chromosome segregation and binucleation in cells from Hutchinson-Gilford progeria patients.<sup>26,27</sup> Thus, it is reasonable to think that a normal growth rate would compromise somatic integrity in progeroid animals. In this case, a metabolic response aimed at reallocating resources from growth to somatic preservation could be the best way to attenuate the consequences of the molecular alterations underlying progeroid syndromes. However, it is clear that this adaptive response fails to counteract the mentioned alterations, which irreversibly lead to the premature death observed in progeroid mice.

The paradoxical finding that autophagy is upregulated in progeroid mice (Fig. 1) may also help to understand the mechanisms underlying the multiple tissue alterations observed in these syndromes. Although the observed metabolic shift could be beneficial, it could also be detrimental for the organism if overactivated (Fig. 2). In this regard, we must take into account the fact that although autophagy activation facilitates temporary adaptation to metabolic stress, this catabolic pathway may also lead to cell death when chronically activated.<sup>28,29</sup> This situation could contribute to the progressive muscular and cardiac wasting observed in both progeroid mice

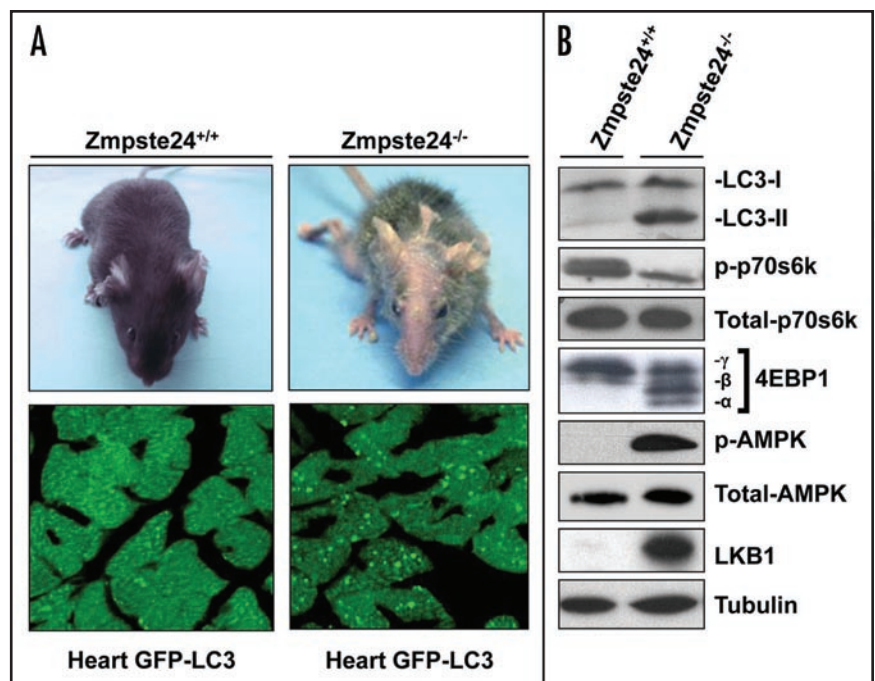


Figure 1. Summary of the most representative autophagy-related alterations observed in progeroid mice. (A) *Zmpste24*<sup>-/-</sup> mice, which show premature aging features as compared to aged-matched *wild-type* littermates (up), present a basal autophagy increase when compared to their *wild-type* littermates (down). (B) Representative immunoblots of the autophagy-related alterations observed in *Zmpste24*<sup>-/-</sup> mice.

and progeria patients.<sup>8,12</sup> However, this hypothesis has to be tested using specific autophagy inhibitors or appropriate animal models of autophagy-deficiency, which are currently unavailable. These further studies will be helpful to clarify whether the observed increase of autophagic activity in progeroid mice helps to slow down the effects of the molecular alterations leading to premature aging, or by contrast, contributes to the development of the multiple pathologies observed in these mice. In this latter case, the autophagy pathway could be a future clinical target which may help to improve the prognosis of progeria patients.

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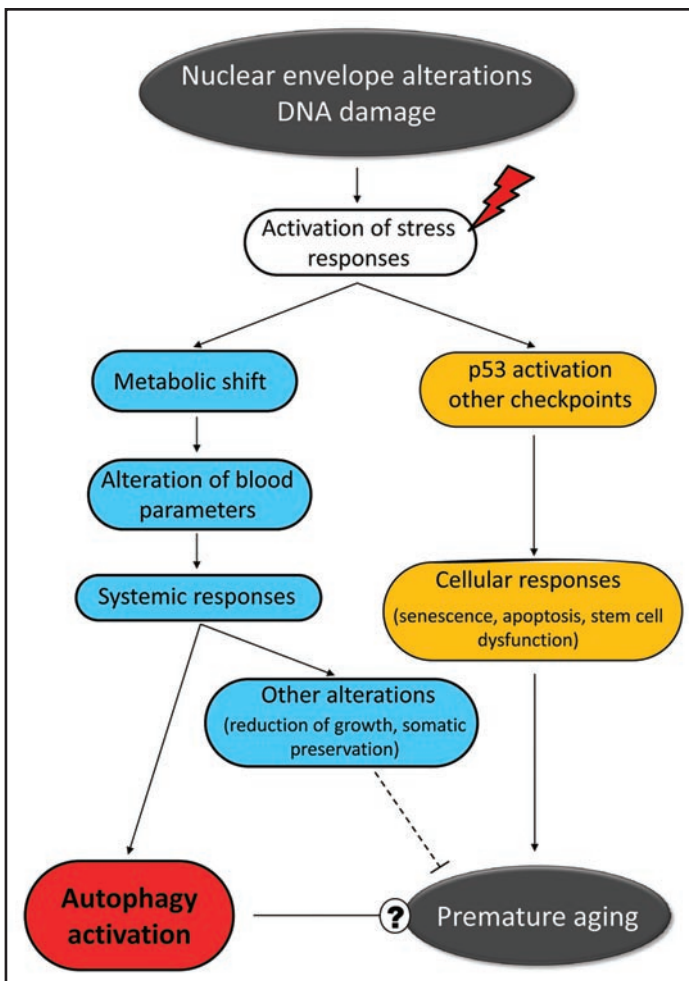


Figure 2. Proposed model for the connections between autophagy and premature aging. The nuclear structure alterations or the accumulation of DNA damage underlying most progeroid syndromes lead to the activation of diverse stress responses, including p53 signaling and stem cell dysfunction, which are both associated with the development of premature aging.<sup>9,30</sup> On the other hand, most changes derived from the metabolic shift observed in progeroid mice probably contribute to slow down the development of aging features.<sup>4,25</sup> However, whether the role played by constitutively activated autophagy is beneficial or detrimental in progeroid syndromes remains unclear.

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