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Enhanced immunity in slowly aging mutant mice with high mitochondrial oxidative stress

Siegfried Hekimi

Department of Biology; McGill University; Montreal, QC Canada

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The mitochondria of slowly aging *Mclk1*$^{+/-}$ mutant mice produce high levels of reactive oxygen species (ROS). These animals display enhanced immune reactivity in response to lipopolysaccharide, *Salmonella*, and tumor-cell grafts, experience limited damage from these treatments and are partially protected from infection and tumorigenesis. We propose that the activation of the immune system by mitochondrial ROS reduces the rate of aging.

In the wild, the intensity of immune responses to pathogens or pre-malignant cells has to be balanced by the need to maintain other activities that are required for survival, such as foraging for food and escaping predators. Caged animals, however, live sheltered lives and might benefit from an enhanced immune reaction. It is interesting to consider this aspect in relationship to the biology of aging. In this context, the immune system has often been considered as the "bad guy." In particular, it is widely believed that inflammation can exacerbate the development of chronic aging-related disorders. Moreover, it has been suggested that episodes of strong inflammation during infancy might lead to detrimental long-term effects because they enhance inflammatory processes later in life.[1] This is based on the observation that, historically, declines in old-age mortality were preceded by declines in early-age mortality due to fewer episodes of severe infection and, thus, inflammation.

MCLK1 is a conserved mitochondrial hydroxylase that is necessary for the biosynthesis of ubiquinone (UQ), also known as coenzyme Q, an electron transporter and mitochondrial membrane antioxidant. The homozygous loss of *Mclk1* is lethal, but the loss of a single copy of the gene leads to reduced levels of UQ in the inner mitochondrial membrane coupled to an unexpected increase of UQ in the outer mitochondrial membrane.[2] This results in reduced rates of electron transport and elevated levels of mitochondrial, but not cytoplasmic, oxidative stress.[3] Surprisingly, this primary phenotype is paralleled by beneficial, rather than detrimental, whole-animal phenotypes, including increased longevity. *Mclk1*$^{+/-}$ mice appear indeed to age slowly as they show a significantly slower increase in biomarkers of aging.[4,5] In addition, *Mclk1*$^{+/-}$ mice are partially protected from the neurotoxic effects of cerebral ischemia-reperfusion and from premature death following spontaneous tumor development in a *Tp53*$^{+/-}$ genetic background.[5,6] These findings, together with cognate observations on the lifespan of *Caenorhabditis elegans*, led us to hypothesize that an increase in the generation of mitochondrial reactive oxygen species (ROS) might accompany aging not because ROS play a causal role in this process but rather because ROS stimulate protective and restorative processes that help to counteract age-dependent damage.[7]

In our search for the mitochondrial ROS-sensitive pathways that might operate in *Mclk1*$^{+/-}$ mice we focused first on the stabilization of the transcription factor hypoxia-inducible factor 1α (HIF-1α), mediating a well-known cytoprotective signal transduction pathway that is regulated by mitochondrial ROS generation.[8,9] Our in vitro, ex vivo, and (limited) in vivo evidence suggests that this pathway is indeed upregulated in *Mclk1*$^{+/-}$ mice.[10]

Among multiple activities, HIF-1α operates as a modulator of the immune response in both the innate and adaptive immune systems. In *Mclk1*$^{+/-}$ mice, peritoneal macrophages were activated along the inflammatory pathway, an effect that depended on mitochondrial ROS as it was suppressed by a mitochondrially-targeted antioxidant peptide.[10] We also found that the elevation of circulating cytokines that accompanies the administration of lipopolysaccharide or infection with *Salmonella* is greatly exaggerated in *Mclk1*$^{+/-}$ animals as compared with their wild-type counterparts.[5,10]

We wondered whether such an altered immune response was in fact beneficial and could thus contribute to the slowly aging phenotype of *Mclk1*$^{+/-}$ mice. Three experimental approaches suggested that this is indeed the case. First, we administered *Salmonella typhimurium* to 129S6 mice, generating a chronic liver infection. We found that 40 days after infection, the liver bacterial load was slightly lower in *Mclk1*$^{+/-}$ mice than in wild-type animals and, interestingly, the molecular and tissue markers of damage that accompany infection and the immune response were strikingly reduced. In particular, we observed reduced levels of oxidative damage to

Correspondence to: Siegfried Hekimi; Email: siegfried.hekimi@mcgill.ca
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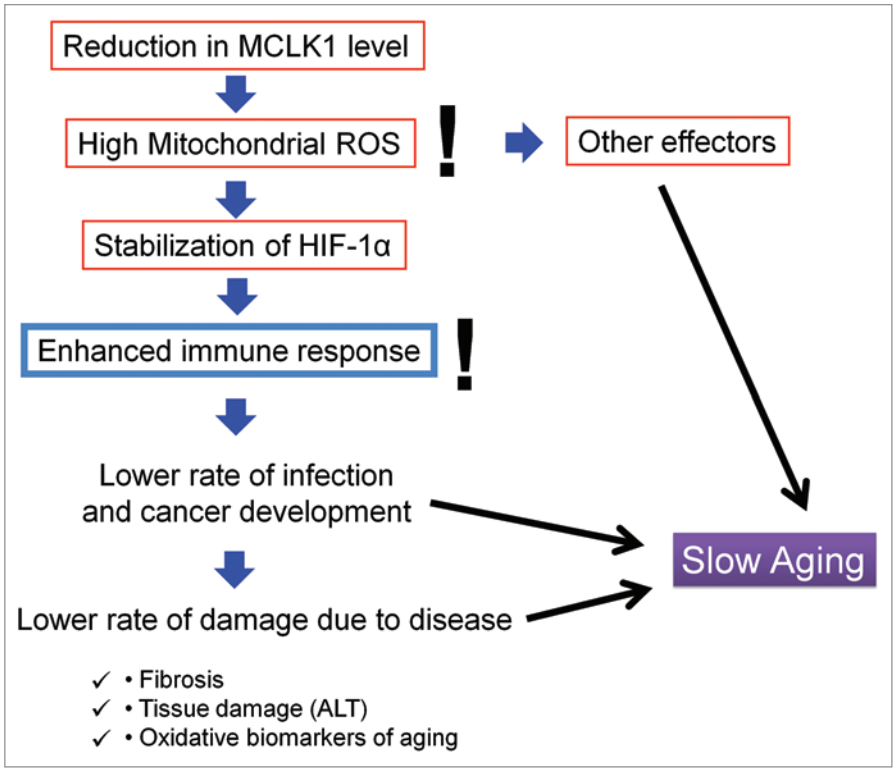

Figure 1. Beneficial impact of mitochondrial ROS on aging. ALT, alanine transferase; HIF-1α, hypoxia-inducible factor 1α; ROS, reactive oxygen species.

proteins and to DNA as well as a reduced level of hepatic fibrosis and of circulating alanine transferase (ALT), a marker of hepatotoxicity, and of hepatic fibrosis. Second, we administered *Salmonella enteritidis* to C57BL/6J mice, which produces a transient liver infection that is fully cleared after ~40 days. We observed no difference in the rate of clearance between *Mclk1*$^{+/-}$ and wild-type mice, but the former manifested lower elevations in plasma ALT, and reduced levels of hepatic fibrosis than the latter, even after three consecutive rounds of infection and clearance. Third, we subcutaneously grafted isogenic 3LL tumor cells into *Mclk1*$^{+/-}$ mice and wild-type littermate controls. The latency before any visible tumor growth was significantly longer in *Mclk1*$^{+/-}$ animals than in wild-type mice, and the cytotoxicity of the splenic T cells of the former against 3LL cells in vitro was significantly enhanced, suggesting that such an increased latency stemmed from a more robust immune response against tumor cells.

These observations are compatible with a model according to which one of the mechanisms by which elevated mitochondrial ROS can exert beneficial effects is by triggering HIF-1α signaling, which in turn stimulates the immune system to provide improved protection against infective and neoplastic challenges thus reducing molecular damage (**Fig. 1**). Thus, we propose that an enhanced activation of the immune system may have long-term effects that are beneficial for lifespan. This provides an alternative interpretation of the observation that people that have not been sick as children live longer.[1] Our findings suggest that rather than being protected from the long-term consequences of early inflammation, individuals with strong immune systems, perhaps due to an optimal nutrition or other favorable environmental factors, are protected from both sickness in childhood and from some of the deleterious consequences of aging later in life. We hope to test this model further by learning to stimulate mitochondrial ROS generation or the immune function by means other than *Mclk1* heterozygosity and to determine whether these interventions also slow down aging.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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