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A novel role for the mitochondrial HTRA2/OMI protease in aging

Seokwon Kang, Teresa Fernandes-Alnemri and Emad S. Alnemri*
Department of Biochemistry and Molecular Biology; The Kimmel Cancer Center; Thomas Jefferson University; Philadelphia, PA USA

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*Correspondence to: Emad S. Alnemri;
Email: Emad.Alnemri@jefferson.edu

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HTRA2/OMI is an ATP-independent serine protease located in the intermembrane space of the mitochondria and is thought to function as a protein quality control protease. Our previous studies showed that loss of the enzymatic activity of HTRA2 due to a Ser276Cys missense mutation in its catalytic domain is associated with early onset neurodegeneration, multiple tissue atrophy and premature lethality in homozygous *htra2*^{mnd2} mice, suggesting that HTRA2 is neuroprotective. To further investigate the role of HTRA2 in neuronal cell survival and the impact of its loss of function in non-neuronal tissues of adult mice, we generated transgenic *htra2*^{mnd2} mice expressing a neuron-targeted human *HTRA2* transgene. Notably, this *HTRA2* transgene rescues *htra2*^{mnd2} mice from early onset neurodegeneration, and other phenotypic abnormalities and prevents their early death, indicating that HTRA2 activity in neuronal mitochondria is important for neuronal cell survival. However, as the rescued *htra2*^{mnd2} mice grow older they exhibit specific phenotypic abnormalities indicative of premature aging. These include premature weight loss, osteoporosis, lordokyphosis, muscle atrophy, heart enlargement, increased autophagy and reduced life span. There is also a significant increase in the levels of clonally expanded mitochondrial DNA (mtDNA) deletions in their tissues. Our findings suggest that HTRA2-regulated protein quality control in the intermembrane space of mitochondria is important for the maintenance of mitochondrial homeostasis, and loss of HTRA2 activity can lead to both neurodegeneration and aging.

The exact function of the mammalian HTRA2 protease has not been fully defined. Earlier studies suggested that HTRA2 functions in apoptosis since it is released from mitochondria together with other apoptotic proteins, such as cytochrome c, and because of its ability to bind and inhibit the activity of inhibitors of apoptosis proteins (IAPs). However, genetic studies in mice do not support a role for HTRA2 in apoptosis. On the contrary, mice lacking HTRA2 activity due to missense mutation (*htra2*^{mnd2} mice) or deletion of the *HtrA2* gene (*htra2* knockout mice), die prematurely as a result of early onset neurodegeneration due to mitochondrial dysfunction and increased sensitivity to stress-induced cell death. Clues to the possible function of HTRA2 came from its crystal structure, which revealed that the structure of HTRA2 is highly similar to that of the bacterial quality control proteases DegP and DegS, suggesting that it too might play a similar role in the mitochondria by degrading misfolded and damaged proteins. Indeed, recent studies with isolated mitochondria from *htra2* knockout mice show increased accumulation of unfolded subunits of respiratory complexes I–IV and generalized respiratory chain dysfunction. Together with the structural studies, these observations suggest that HTRA2 is a protein quality control protease important for mitochondrial homeostasis.

To gain more insights into the function of HTRA2 and whether it plays a protective role in non-neuronal tissues, we generated transgenic *htra2*^{mnd2} mice that are deficient in HTRA2 activity in non-neuronal tissues but now express functional

neuron-targeted human *HTRA2* transgene in their neurons. Interestingly, expression of this *HTRA2* transgene in neurons rescues *htra2*^{mnd2} mice from rapid onset neurodegeneration, multiple tissue atrophy and early lethality. Although rescued *htra2*^{mnd2} mice are phenotypically indistinguishable from wild-type mice at 4–5 mo of age, they begin to show signs of premature aging as they get older and die between 12–17 mo of age. Of particular interest is the presence of clear pathological features of cardiac aging, as all aged rescued *htra2*^{mnd2} mice show obvious heart enlargement with left ventricular hypertrophy. This is associated with decreased glucose metabolism, increased mtDNA deletions and increased autophagosome activity in heart tissues. The increased autophagosome activity is likely due to increased mitophagy, as our in vitro experiments revealed an increase in the degradation rate of two mitochondrial proteins, PPID/peptidylprolylisomerase D (cyclophilin D) and SOD2/MnSOD, in response to starvation in HTRA2-deficient MEFs compared with HTRA2-expressing MEFs. The mechanism by which loss of HTRA2 activity in cardiac cells can lead to cardiac aging is not clear at present, but we can speculate that perturbations in protein quality control in the intermembrane space of the mitochondria because of HTRA2 deficiency might lead to the disassembly and aggregation of respiratory complexes over time, as a result of reactive oxygen species (ROS) production during oxidative phosphorylation. As a consequence, ROS production is expected to further rise, which increases the rate of mtDNA mutations and deletions leading to further deterioration in the function of the respiratory complexes. Eventually, these changes activate the cellular autophagy machinery to remove the dysfunctional mitochondria by mitophagy. We propose that the increased autophagosome activity and mitophagy in HTRA2-deficient cells might be responsible for the observed clonal expansion of mitochondria with large DNA deletions that span most of the coding regions for the mitochondria-encoded respiratory chain subunits. Since ROS is a major signal that activates mitophagy through the PINK1-PARK2 pathway, we speculate that mitochondria with large DNA deletions, like mitochondria that lack mtDNA, are incapable of generating ROS and therefore will escape mitophagy. Because increased mitophagy is usually compensated for by increased mitochondrial biogenesis to meet the energetic demand in somatic cells, those mitochondria with large mtDNA deletions that escaped mitophagy would undergo clonal expansion. Since these mitochondria are less efficient in producing ATP, they will not be able to meet the high energy demands of tissues like cardiac muscles, thereby leading to organ failure as observed in rescued *htra2*^{mnd2} mice and mtDNAmutator mice.

Collectively, our findings offer the first genetic evidence that perturbations in protein quality control in the intermembrane space of the mitochondria due to loss of HTRA2 activity can lead to age-related diseases. These diseases might be the outcome of increased mtDNA deletions and clonal expansion of mitochondria harboring these deletions, which reduce mitochondrial energy production, thus compromising cellular and organ functions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.