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Caspase-2 and the oxidative stress response

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Caspase-2, one of the earliest discovered caspases, has emerged as a multifunctional enzyme with roles that are not limited to cell death. It acts as a tumor suppressor, prevents genetic instability, and protects against aging by playing a crucial role in sensing alterations in cellular redox status and activating the antioxidant defense system. These apparent non-apoptotic functions, only discovered recently, emphasize the importance of this often-neglected protease.

Since its discovery almost 2 decades ago,[1] caspase-2 has intrigued the scientific community. It is activated in response to diverse apoptotic stimuli both upstream and downstream of mitochondrial outer membrane permeabilization (MOMP) and, unlike many other proteins, can initiate cell death even in the absence of reported adaptor proteins such as p53-induced protein with a death domain (PIDD) and RIP-associated ICH-1 homologous protein with a death domain (RAIDD). Furthermore, caspase-2 has been implicated in several other roles including tumor suppression, maintenance of genetic stability, autophagy, and aging.[2,3]

Recent studies from our laboratory indicate that caspase-2 knockout (*Casp2*$^{-/-}$) mice have a reduced maximum lifespan and show early onset of several aging related traits.[4] This was in agreement with a previous study using *Casp2*$^{-/-}$ mice that were generated independently.[5] Aged *Casp2*$^{-/-}$ mice had reduced body weight and bone volume and showed early hair graying and reduced subcutaneous fat thickness.[4] Interestingly, no spontaneous tumor formation was evident as these mice aged. However, several aged *Casp2*$^{-/-}$ mice developed splenomegaly and had prominent lymphocytic infiltration in the liver.[4] One of our key observations was that aged *Casp2*$^{-/-}$ mice experienced oxidative stress and showed evidence of increased oxidative DNA damage in the liver. The activity of the antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) was reduced with a concomitant increase in protein and lipid oxidation. In order to understand this further we used ethanol as an oxidative challenge. Adult mice were fed a diet containing ethanol, and both wild-type (WT) and *Casp2*$^{-/-}$ mice showed increased steatosis and liver damage. Interestingly, *Casp2*$^{-/-}$ mice did not show any increase in SOD and GSH-Px activity. These results suggest that caspase-2 acts as a cellular sensor for reactive oxygen species (ROS). In the absence of caspase-2, cells fail to respond to exogenous oxidative challenge by upregulating the protective antioxidant mechanisms.[4] This results in more oxidative damage in *Casp2*$^{-/-}$ mice and the prolonged exposure to stress causes accumulation of damaged cells, leading to the functional decline that is often associated with aging. We extended these observations using another robust free radical generator, paraquat (PQ).[6] Paraquat is a widely-used superoxide radical generator that primarily affects the lungs. In this study we included a cohort that received the antioxidant, N-acetyl cysteine (NAC). NAC was administered prior to PQ injection and the mice had *ad libitum* access for the entire 2-week observation time post PQ injection. We observed that pulmonary lesions in *Casp2*$^{-/-}$ mice were consistently more severe than those in WT mice. Caspase-2 was activated in response to PQ, and NAC prevented its activation. However, even though caspase-2 was not activated, cellular damage occurred in the NAC supplemented group and only partial rescue was observed by histology of lung and liver tissue.[6] It is important to highlight that a very mild dose of PQ (15 mg/kg body weight) was used because higher doses are fatal. The benefit of a low dose is that it allows time to monitor animal heath (such as acute body weight loss or respiratory distress), which was not different between the 2 genotypes. In addition, subtle differences are difficult to demarcate with a high dose that causes extensive damage. Similar to our previous observations, induction of SOD and GSH-Px was not observed in PQ-treated *Casp2*$^{-/-}$ mice.[6] Expression of forkhead homeobox type O (Foxo), SOD2, and nuclear factor erythroid 2-related factor 2 (Nrf2) was significantly reduced in the lungs of *Casp2*$^{-/-}$ mice. The two major observations were: (1) PQ-induced stress caused karyomegaly and binucleation in *Casp2*$^{-/-}$ mouse liver in a dose-dependent manner. Although the liver is not the primary target tissue, it is the seat of metabolism where the effects on nuclear size were easy to identify; and (2) Caspase-2 prevented the inflammatory response as serum IL6 and IL-1β levels were higher in

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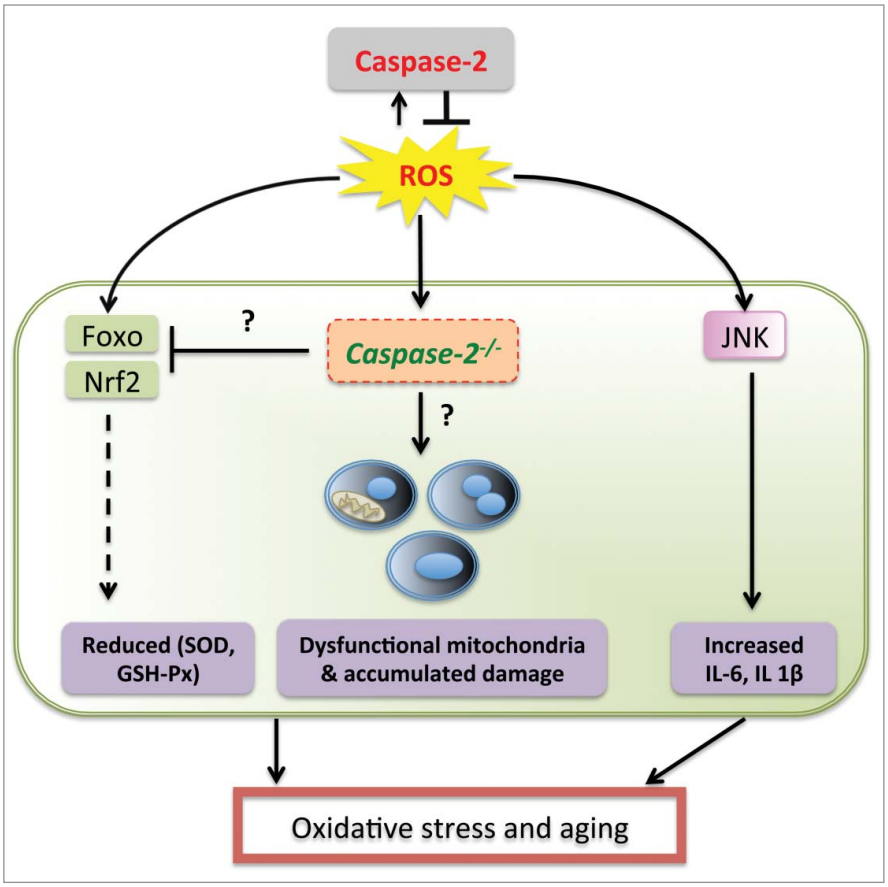

Figure 1. Possible role of caspase-2 in protecting against oxidative stress. Caspase-2 is normally activated in response to oxidants/reactive oxygen species (ROS), thus preventing oxidative stress. In cells lacking caspase-2, the threshold to withstand stress is reduced and accumulation of cells with dysfunctional mitochondria, binucleation, and karyomegaly is often observed, perhaps as a result of perturbed cell cycling. Expression of the antioxidant responsive genes *Foxo* and *Nrf2* is reduced and no increase in SOD and GSH-Px enzyme activity occurs to counteract the oxidative challenge. As a result there is increased oxidative damage to proteins and DNA, and such cells produce more ROS. This activates other redox-sensitive proteins such as JNK, increasing the secretion of some inflammatory cytokines. Thus, increased oxidative stress probably causes the aging phenotype observed in *caspase-2* knockout mice.

PQ-injected *Casp2*$^{-/-}$ mice compared to WT.[6]

Loss of caspase-2 increases genomic instability and predisposes mice to thymomas, lymphomas, and MMTV-induced mammary tumors, which display karyomegaly and aberrant mitosis (reviewed in ref.[7]).[7,8] The fact that *Casp2*$^{-/-}$ mice develop cell cycle defects earlier and in response to low doses of the stressor strengthens our hypothesis that *Casp2*$^{-/-}$ mice have reduced stress tolerance and a compromised background that makes them more susceptible to various oxidative and oncogenic stimuli. This may at least partially account for the early aging phenotype in these mice.

Surprisingly, we also observed increased Jun N-terminal kinase (JNK) activation without any increased cell death in PQ-treated *Casp2*$^{-/-}$ mice. The increase in JNK activity may be an adaptive response in cells lacking caspase-2 (*i.e.*, these cells rely on another stress sensor) and/or may simply indicate higher ROS production/oxidative stress in these cells, which forces activation of other redox sensitive pathways even under mild stress whereas it evokes no such response in WT mice. It is very likely that increased stress in *Casp2*$^{-/-}$ mice independently activates JNK, which may prevent oxidative stress while also activating the inflammatory response that would account for the elevated IL6 and IL-1β levels in *Casp2*$^{-/-}$ mice.[6]

To identify the cause of the increased ROS, we analyzed mitochondria, the chief cellular ROS generators. Hepatic mitochondria from 12-month-old *Casp2*$^{-/-}$ mice (middle aged) were identical in performance to those from 24-month-old WT mice (old); moreover, the *Casp2*$^{-/-}$ mice had increased complex III activity.[9] Using several distinct approaches, caspase-2 has been implicated as a fine-tuner of several metabolic processes during aging. Several enzymes involved in carbohydrate metabolism are upregulated, whereas ribosomal and respiratory complex proteins are downregulated.[10] Similar to the previous study, the metabolic profile changes occurring in the young *Casp2*$^{-/-}$ mice resemble those found in aged WT mice, indicating that mitochondrial dysfunction is another likely contributor to premature aging in *Casp2*$^{-/-}$ mice.

In the absence of unique caspase-2 substrates involved in redox regulation, is it not possible to pinpoint the exact mechanism by which caspase-2 prevents oxidative damage, although some affected proteins/enzymes/pathways have now been identified (**Fig. 1**). Nevertheless, the protective roles of caspase-2 in oxidative stress management and prevention of aging are now clearly established by the recent i*n vivo* studies summarized in this article.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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