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Putative functions of caspase-2

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Abstract

Caspase-2 is the most evolutionarily conserved of caspase family members, yet its physiological function has remained unclear and is a matter of considerable debate. Newly published data now suggest that caspase-2 is required for cell cycle regulation, repair of damaged DNA, and in suppressing Myc-induced lymphomagenesis. Additionally, loss of *Casp2* in mice leads to features of premature ageing. These findings suggest that caspase-2 has non-apoptotic functions in addition to its context-dependent roles in cell death.

Introduction and context

Caspase-2 is one of the closest mammalian homologues of the *Caenorhabditis elegans* caspase CED-3 and shares significant homology with the *Drosophila* Nedd2-like caspase (DRONC), both of which are essential for developmentally programmed cell death [1-4]. Although several studies have implicated caspase-2 as a crucial mediator of apoptosis in mammalian cells, its apoptotic function has remained enigmatic, partly due to the fact that *Casp2*$^{-/-}$ mice are viable and fertile with only minor apoptotic defects in some cell types [2,5-7]. Furthermore, lymphocytes and fibroblasts from mice lacking both initiator caspases, *Casp9* and *Casp2*, are no more resistant to apoptosis than cells from *Casp9*$^{-/-}$ mice [8]. Together, these findings indicate that the role of caspase-2 in developmental cell death is redundant and can be compensated by other caspases. However, this does not rule out context-dependent and cell-specific caspase-2 functions. For example, one study found an accumulation of oocytes in *Casp2*$^{-/-}$ mice (although this was not reported in a second *Casp2*$^{-/-}$ strain) and *Casp2*$^{-/-}$ mice display premature ageing-related traits [5,9,10].

The activation of caspase-2 has been shown to occur both upstream (by the PIDDosome) and downstream (by caspase-3 or -7) of mitochondrial outer membrane permeabilisation (MOMP) [11,12]. Although this is also controversial since RAIDD (receptor-interacting protein-associated ICH-1/CED-3 homologous protein with a death domain) and PIDD (p53-inducible protein with a death domain), the protein components of the PIDDosome, are dispensable for caspase-2 activation [13,14]. Interestingly, caspase-2 activation can be mediated by caspase-8-induced cleavage following recruitment to the death receptor-inducing signaling complex (DISC) [15]. However, the importance of the DISC as an activation platform is also unclear since caspase-2 dimerisation and self-processing are sufficient for its activation [14,16]. In addition, cells from *Casp2*$^{-/-}$ mice are normally sensitive to death receptor-induced apoptosis [6], indicating that caspase-8-mediated cleavage of caspase-2 is not critical for its activation. While there is limited information on physiologically relevant substrates, caspase-2 can cleave and activate the protein Bid, which provides a significant link between caspase-2 and MOMP [15,17]. Furthermore, a unique feature of caspase-2 is its ability to localise to the nucleus in an importin-mediated fashion [18-20]. This nuclear localisation of caspase-2 is likely associated with the recently found functions for caspase-2 in cell cycle regulation and cellular DNA damage response.

Major recent advances

In the absence of an overt phenotype in knockout mice, one may speculate that caspase-2 functions under specific contexts, such as under conditions of stress or in the

fine-tuning of stress signaling, resulting in relatively minor aberrations in the whole animal physiology. Several recent studies showing caspase-2 functions in cell cycle regulation, DNA damage response, and tumor suppression seem to be consistent with these predictions.

A role for caspase-2 in cell cycle regulation became apparent from observations that *Casp2*-deficient murine embryonic fibroblasts (MEFs) proliferate faster than their wild-type counterparts and that transformation of *Casp2*$^{-/-}$ MEFs with E1A/Ras exacerbated this proliferative effect [21]. Another recent study found that caspase-2 is involved in maintaining a G_2/M cell cycle checkpoint in response to ionising radiation (IR)-induced DNA damage, with cells lacking *Casp2* unable to completely arrest in G_2/M [21,22]. In addition, caspase-2 activation has been shown to be inhibited by cyclin-dependent kinase 1 (Cdk1)/cyclin-B1-mediated phosphorylation at Ser340 during mitosis to allow for the repair of replication-induced DNA damage [23]. During mitotic arrest, prolonged activation of spindle assembly checkpoint results in apoptosis by 'mitotic catastrophe', which may be mediated by caspase-2 [23,24]. When caspase-2 is lacking, arrested cells cannot undergo apoptosis but instead undergo 'mitotic slippage' and prematurely exit mitosis with chromosomal abnormalities, resulting in genomically unstable aneuploid cells [23]. In support of this, *Casp2*$^{-/-}$ MEFs show resistance to cell death induced by microtubule-disrupting drugs and also display increased genomic instability in culture compared with wild-type cells [7,21]. These findings indicate that deregulation of G_2/M checkpoint in *Casp2*$^{-/-}$ cells may contribute to the accumulation of cells with damaged DNA.

The study by Shi and colleagues [22] found that caspase-2 is involved in DNA damage repair through its interaction with a nuclear complex comprising PIDD and DNA-dependent protein kinase catalytic subunit (DNA-PKc). Following IR-induced DNA damage, this DNA-PKc PIDDosome complex phosphorylates caspase-2 at Ser122, leading to its activation. Activated caspase-2 is then required for the repair of double-strand DNA breaks by non-homologous end-joining (NHEJ) with cells lacking *Casp2* unable to efficiently repair DNA breaks [22]. Although it is unclear how caspase-2 mediates NHEJ, these important observations establish an additional non-apoptotic nuclear role for caspase-2 in DNA damage signaling (Figure 1).

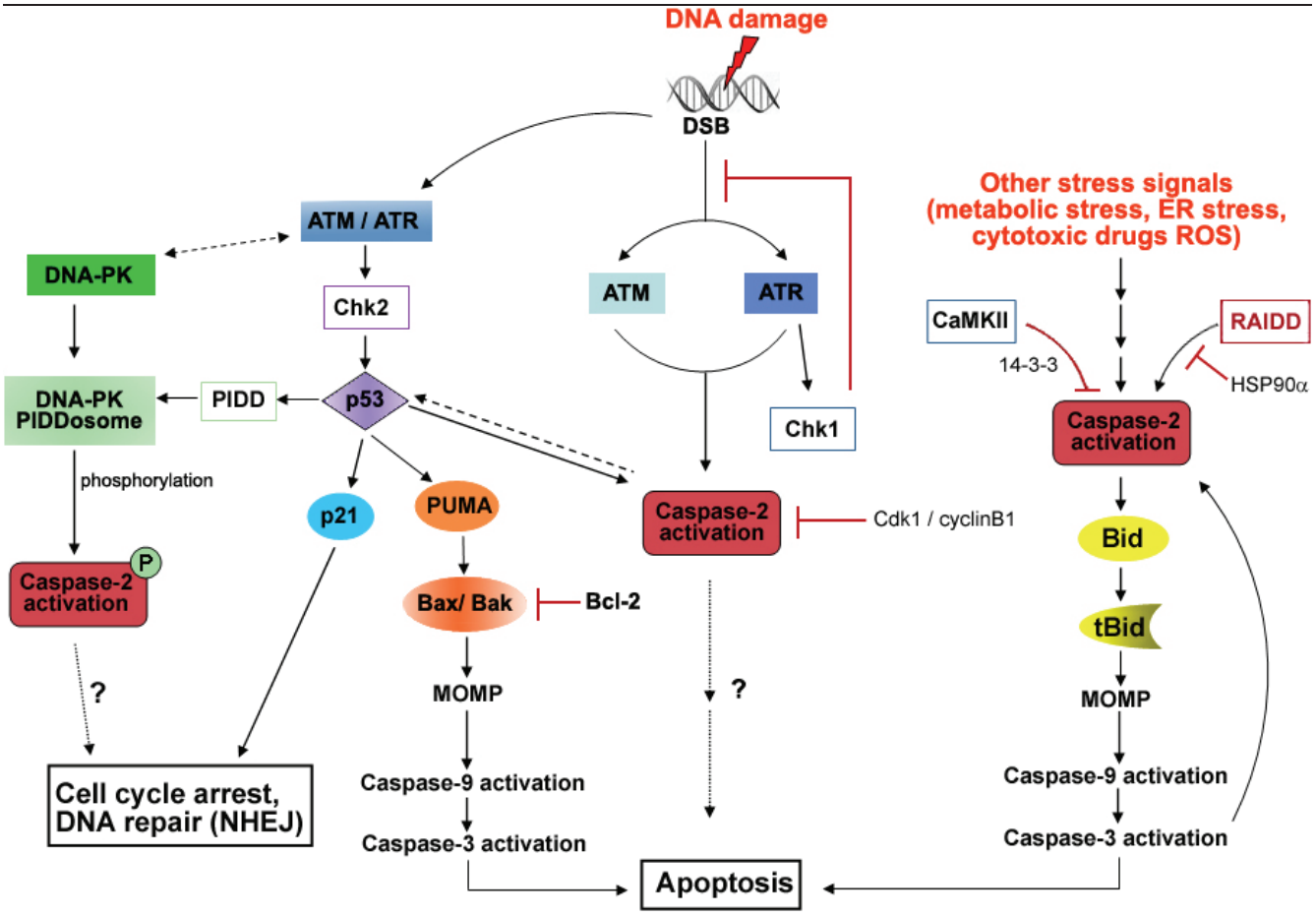
Sidi and colleagues [25] have described an unexpected nuclear function of caspase-2 in an apparently novel pathway of apoptosis in *p53*-deficient cells. Using zebrafish as a model system, it was found that inhibition or loss of checkpoint kinase 1 (Chk1) restores γ-radiation-induced apoptosis in *p53* mutant fish embryos [25]. A similar ataxia telangiectasia mutated (ATM)/ATM-related (ATR)/caspase-2-dependent pathway seems to be present in Chk1-inhibited p53-deficient human tumor cells and in MEFs following γ-irradiation [21,25]. These findings implicate caspase-2 in an apoptosis pathway downstream of ATM/ATR in response to DNA damage, which is independent of p53 (Figure 1). Since ATM/ATR can induce phosphorylation and activation of DNA-PKc, it would be of interest to investigate whether the regulation of caspase-2 phosphorylation in the nucleus finely tunes its function in either apoptosis or DNA damage repair following IR exposure.

The role of caspase-2 in specific cell death pathways other than DNA damage is also emerging. In oocytes, the apoptotic activity of caspase-2 has been shown to be inhibited by Ca^{2+}/calmodulin-dependent kinase II (CaM-KII)-mediated phosphorylation at Ser135 [26]. The binding of 14-3-3ζ to phosphorylated caspase-2 prevents Ser135 dephosphorylation, thereby promoting oocyte survival [27]. However, under nutrient-depleted conditions, the dephosphorylation at this site leads to caspase-2 activation and oocyte cell death [26]. These findings suggest that caspase-2 is an important player in metabolic regulation of oocyte cell death and that Ser135 dephosphorylation is a sensor for caspase-2 activation.

Caspase-2 has also been implicated in cell death induced by heat shock [28]. A recent study assessed the real-time recruitment of caspase-2 to activation platforms during stress-induced apoptosis, including heat shock, cytoskeletal disruption or DNA damage, and found that caspase-2 activation occurred in the cytosol, not the nucleus [29]. Furthermore, heat shock-induced activation of caspase-2 occurred upstream of MOMP and was RAIDD-dependent and negatively regulated by HSP90α [29].

The caspase-2 functions in oxidative stress-induced apoptosis and ageing are also coming to light. Caspase-2, along with Bid and Bak, were reported to be mediators of superoxide-induced cell death in muscle and in primary neurons [30,31]. In addition, caspase-2 has been shown to be involved in an age-related increase in muscle cell apoptosis in mice [9]. Consistent with these findings, Zhang and colleagues [10] found that *Casp2*$^{-/-}$ mice show significantly higher levels of oxidised proteins in liver than wild-type mice. This indicates that lack of *Casp2* can antagonise apoptosis induced by reactive oxygen species, leading to accumulation of cells with oxidative damage and consequently enhanced ageing phenotypes [10]. The reduced NHEJ activity in *Casp2*$^{-/-}$ cells may also contribute to the premature ageing phenotype observed in *Casp2*$^{-/-}$ mice.

Figure 1. Putative functions of caspase-2



Following double-strand DNA breaks (DSBs), the ataxia telangiectasia mutated (ATM) and ATM-related (ATR) kinases are activated and in turn phosphorylate and activate several target proteins, including checkpoint kinase 1 (Chk1) and Chk2. Chk2 activates the p53 response pathway, which can lead to cell cycle arrest. ATR activation leads to the activation of caspase-2 and apoptosis following irreparable DNA damage. ATR also activates Chk1, which can then act in a feedback loop to negatively regulate ATR and inhibit further activation of nuclear caspase-2. DNA-dependent protein kinase (DNA-PK) is also activated by DSBs, presumably by ATM/ATR, and forms a complex with p53-inducible protein with a death domain (PIDD) and caspase-2 (DNA-PK PIDDosome). This complex serves to phosphorylate and activate caspase-2, which is then required for the initiation of non-homologous end-joining (NHEJ) and DNA repair. Cytosolic caspase-2 is also activated by other stress signals such as reactive oxygen species (ROS), metabolic stress, cytotoxic drugs, heat shock, or endoplasmic reticulum (ER) stress. Following heat shock, RAIDD (receptor-interacting protein-associated ICH-1/CED-3 homologous protein with a death domain) can activate caspase-2, which is inhibited by HSP90α. Ca^{2+}/calmodulin-dependent kinase II (CaMKII) acts to inhibit caspase-2 activation and cell death in oocytes. Activated cytosolic caspase-2 is able to cleave Bid to its truncated form (tBid), which (via Bax/Bak) can induce mitochondrial outer membrane permeability (MOMP), activation of caspase-9 and -3, and cell death. P, phosphorous; PUMA, p53-upregulated mediator of apoptosis.

Our own studies using the Eμ-Myc transgenic mouse model of B-cell lymphoma found a potential role for caspase-2 in lymphoma suppression [21]. Specifically, the loss of even a single copy of *Casp2* resulted in increased tumor susceptibility and markedly accelerated tumor formation in Eμ-Myc transgenic mice [21]. These studies suggest that caspase-2 can suppress Myc-induced lymphomagenesis. While the precise mechanism of caspase-2-induced tumor suppression remains unclear, it is tempting to speculate that its roles in cell cycle checkpoint, DNA damage repair, and removal of oxidative damaged cells are important for this function.

Future directions

While the recent observations have shed light on possible physiological functions of caspase-2, it remains entirely speculative how caspase-2 might carry out some of the apparently unrelated functions in apoptotic and

non-apoptotic contexts. It is becoming clear that caspase-2 may act as a sensor to protect against cellular stress and that regulation of caspase-2 by phosphorylation or nuclear translocation or both may determine its role in cell death, the cell cycle, or NHEJ. It will be important to establish whether these functions also contribute to the tumor suppressor mechanism of caspase-2. The major deficiency in caspase-2 research is that the targets of caspase-2, which may mediate its various functions, remain largely unknown. While it remains a technical challenge to find proteins that are specifically cleaved by caspase-2 in specific contexts, identification of these substrates will be the key to unraveling the functional versatility of caspase-2.

Abbreviations

ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia mutated-related; CED-3, CEll Death abnormality 3; Chk1, checkpoint kinase 1; DISC, death receptor-inducing signaling complex; DNA-PKc, DNA-dependent protein kinase catalytic subunit; IR, ionising radiation; MEF, murine embryonic fibroblast; MOMP, mitochondrial outer membrane permeabilisation; NHEJ, non-homologous end-joining; PIDD, p53-inducible protein with a death domain; RAIDD, receptor-interacting protein-associated ICH-1/CED-3 homologous protein with a death domain.

Competing interests

The authors declare that they have no competing interests.

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