

Nucleolar caspase-2: Protecting us from DNA damage

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Caspase-2 triggers apoptosis, but how it is activated by different stimuli is unclear. In this issue, Ando et al. (2017. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201608095>) delineate two pathways of caspase-2 activation and show that, in response to DNA damage, caspase-2 forms a complex with the PIDDosome and NPM1 within the nucleolus.

Caspase-2 is a critical component of the cellular machinery designed to remove damaged cells, preventing disease. Consistent with a function in the apoptosis pathway, caspase-2, like caspase-3, is a tumor suppressor (Puccini et al., 2013). Although caspase-2 is required to initiate an effective apoptotic response following stimuli, depletion of caspase-2 has multiple cellular consequences, such as genomic instability, altered cell cycle regulation, activation of the oxidative stress response, and premature ageing (Dorstyn et al., 2012; Shalini et al., 2012). Interestingly, all of these pathways are also implicated in the development and evolution of cancer. Indeed, genomic instability is a universal hallmark of cancer and the induction of genomic instability by depletion of caspase-2 suggested that the DNA damage response pathway signals through caspase-2. p53 is an important responder to genomic instability and caspase-2 is activated after genomic stress, in both a p53-dependent and -independent manner (Lassus et al., 2002; Sidi et al., 2008).

The evolutionary conserved caspase-2 is important in signaling cell death, but how caspase-2 responds to different death stimuli is not clear. Caspase-2, like caspase-9, caspase-8, and caspase-1, is considered an initiator caspase, with an apical position in signaling of the stress stimuli. Some stimuli trigger caspase-2 activation through the PIDDosome complex—a high-molecular weight protein assembly that is well characterized to be an “activation platform” for caspase-2 and contains the PIDD1 and RAIDD proteins (Tinel et al., 2007). Although it was initially thought to be a DNA damage–responsive complex, loss of the PIDDosome function in mice does not impair the DNA damage response, DNA damage-induced apoptosis, or DNA damage-induced tumor formation. The exact role of the PIDDosome is still unclear. Additionally, it was shown that caspase-2 is activated in a PIDDosome-independent mechanism in response to other stimuli (Bouchier-Hayes et al., 2009), raising the possibility that caspase-2 is involved in different cellular pathways. In new research published in this issue, Ando et al. used a proximity fluorescence caspase-2 activation reporter to show that different caspase-2 activation platforms assemble in

the cytoplasm and in the nucleolus and their requirement for PIDDosome proteins differs.

Ando et al. (2017) determined that different caspase-2–activating stimuli had different cellular consequences for caspase-2. The authors demonstrated that DNA-damaging agents, such as topoisomerase I inhibitors, topoisomerase II inhibitors, camptothecin, irinotecan, and topotecan, resulted in a predominantly nucleolar activation of caspase-2, whereas treatment with the microtubule inhibitor vincristine resulted in cytoplasmic activation of caspase-2. The researchers were able to decipher which stimulus required nucleolar localization of caspase-2 for caspase-2 activation using mutant constructs with and without the nuclear localization sequence of caspase-2 and 3D imaging. They found that cytoplasmic activation of caspase-2 using heat shock did not require the nuclear localization sequence, whereas nucleolus activation of caspase-2 by DNA damage did. This data supported the hypothesis that caspase-2 had two modes of activation, one based on a response to genotoxic agents that is confined in the nucleolus and the other in response to other stress stimuli, occurring in the cytoplasm and nucleus.

To understand this mechanism further, Ando et al. (2017) used immunofluorescence microscopy to study the behavior of caspase-2 after various stimuli in more detail. After nongenomic stress, with vincristine and etoposide, the formation of predominantly cytoplasmic caspase 2 puncta was observed, whereas treatment with DNA-damaging agents resulted in predominantly large nucleolar puncta. The nucleolar puncta suggested that caspase-2 localized to a specific nucleolar substructure. There are three molecularly defined nucleolar substructures: the fibrillar center, the dense fibrillar center, and the granular component. Localization studies determined that caspase-2 accumulated predominantly in the fibrillar center, suggesting that caspase-2 activation platforms assemble predominantly in the fibrillar center, with some localization at the internal boundary of the granular component. Intriguingly, Ando et al. (2017) also observed that, after DNA damage, the abundant nucleolar protein NPM1 also changed location from the granular component to a ring structure surrounding caspase-2, indicating that NPM1 may be involved in the caspase-2 response.

These data suggest that caspase-2 has two distinct cellular localization and activation profiles. Ando et al. (2017) sought to understand how the PIDDosome functions in this process. The researchers dissected the requirement for each PIDDosome component—PIDD1 and RAIDD—in nucleolar caspase-2

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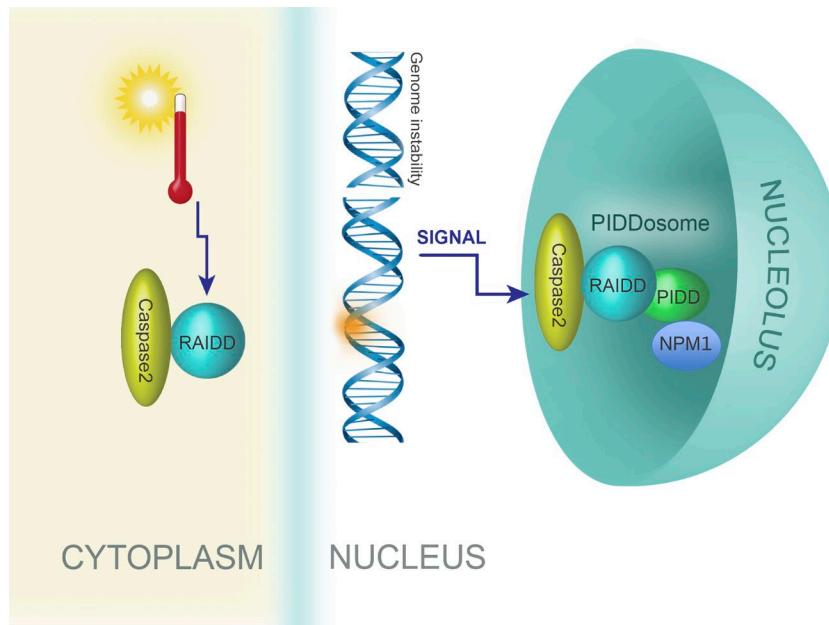


Figure 1. Model representing the two compartments of caspase-2 activation. Caspase-2 activation by DNA damage is primarily localized to the nucleolus and is dependent on the PIDDosome and NPM1. Caspase-2 activation in the cytoplasm by non-DNA damaging stimuli is RAIDD dependent but neither requires NPM1 nor the PIDDosome.

activation using mouse embryonic fibroblasts deficient in either PIDD1 or RAIDD. Interestingly, although RAIDD was required for caspase-2 activation in the cytoplasm nucleus and nucleolus, PIDD was only required for nucleolar caspase-2 activation. Surprisingly, PIDD is thus critical and specific for the DNA damage-induced nucleolar activation of caspase-2.

To further explore the potential contribution of other partners in caspase-2 activation in the nucleolus, the researchers turned their attention to NPM1. NPM1 localized proximal to activated caspase-2 in the nucleolus, so it could hypothetically interact with caspase-2 or the PIDDosome activation platform. Using mass spectrometry, the authors identified an interaction between NPM1 and PIDD. Using pulldown assays, they confirmed this interaction to be damage inducible, with pretreatment of cells with the DNA-damaging agent camptothecin inducing the interaction. However, apoptosis induced by the non-DNA-damaging agent actinomycin D (a microtubule inhibitor) did not induce the interaction. The full-length PIDD polypeptide is constitutively autoprocessed into three fragments termed PIDD-N, PIDD-C, and PIDD-CC. To further understand the functionality of the NPM1–PIDD interaction, Ando et al. (2017) mapped the domain of PIDD required for this interaction, revealing two distinct NPM1 binding sites on PIDD. NPM1–PIDD interactions are mediated by both the PIDD-N and PIDD-CC polypeptides. Whereas the PIDD-CC polypeptide is known to be required for binding to RAIDD (Tinel et al., 2007), the PIDD-N terminal has an uncharacterized regulatory role within the cell. Reciprocal mapping of NPM1 revealed the requirement of the central domain of NPM1 for binding to PIDD. This acidic domain of NPM1 is thought to be required for histone interaction, suggesting a new important role for this domain. Although it is unclear if the interaction between PIDD and NPM1 is direct, these results implicate NPM1 in the nucleolar caspase-2 activation complex. Further, Ando et al. (2017) demonstrated that depletion of NPM1 by siRNA resulted in decreased activated caspase-2 in the nucleolus in response to camptothecin, a similar phenotype to what they observed with PIDD depletion by siRNA. Supporting this observation, NPM1 depletion inhibited caspase-2 cleavage after DNA damage

several cell lines. Thus, NPM1 and the NPM1–PIDD interaction are critical for damage-induced nucleolar PIDDosome signaling. Interestingly, NPM1 itself is known to have roles in apoptosis; in particular, NPM1 is the main cellular reservoir of p14ARF (Box et al., 2016). Further, NPM1 translocates to distinct nuclear foci after induction of DNA damage by ionizing radiation (Koike et al., 2010). It would therefore be interesting to determine if the release of p14ARF from NPM1 is required for the binding of PIDD. Further research will be required to fully unravel the interplay between NPM1, p14ARF, caspase-2, and the DNA damage response. However, it is clear that both NPM1 and caspase-2 have critical roles in the apoptotic response to DNA damage.

NPM1 itself is an important protein in disease. In acute myeloid leukaemia (AML), ~30% of patients have mutations in *NPM1* (affecting the C terminus of the protein in particular; Box et al., 2016). Mutant NPM1 lacks the nuclear localization signal at the C terminus and accumulates in the cytoplasm. Patients with mutant NPM1 respond better to DNA-damaging therapy, probably because of intrinsic genomic instability combined with the extra instability induced by the chemotherapy. Consistent with the critical role of NPM1 in caspase-2 activation, Ando et al. (2017) observed that cells expressing mutant NPM1 do not initiate the caspase-2 nucleolar response to DNA damage. This may explain, in some part, the pathology of this disease. Importantly, the authors demonstrated that *Npm1*^{−/−} mouse embryonic fibroblasts were also highly resistant to apoptosis induced by DNA-damaging agents, which would seem to contradict what is observed in AML. This may be a result of the differences between *in vitro* and *in vivo* conditions. It may also be explained by differences in cellular phenotypes between mutated NPM1 and loss of the NPM1 protein.

In summary, Ando et al. (2017) unravel some of the mysteries surrounding caspase-2 function within the cell (Fig. 1). Their study revealed that DNA damage-induced caspase-2 activation is compartmentalized within the cell, into the nucleolus. Site-specific caspase-2 activation is dependent on the PIDDosome, whereas cytoplasmic and nuclear activation does not depend on this complex. Intriguingly, the authors revealed

that PIDD interacts with NPM1, a step critical for caspase-2 activation within the nucleolus. The study also shed light on the molecular role of NPM1 mutation pathology in AML. Patients with mutations in *NPM1* that result in loss of the nuclear localization sequence and mislocalization of the protein do better than patients with wild-type NPM1. The lack of an efficient caspase-2 apoptotic response allows cells a greater degree of genomic instability, making them potentially more susceptible to chemotherapeutics. The results from Ando et al. (2017) overall highlight the complexity and the importance of caspase-2 function within the cell.

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