

Review Article

PIDD1 in cell cycle control, sterile inflammation and cell death

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The death fold domain-containing protein PIDD1 has recently attracted renewed attention as a regulator of the orphan cell death-related protease, Caspase-2. Caspase-2 can activate p53 to promote cell cycle arrest in response to centrosome aberrations, and its activation requires formation of the PIDDosome multi-protein complex containing multimers of PIDD1 and the adapter RAIDD/CRADD at its core. However, PIDD1 appears to be able to engage with multiple client proteins to promote an even broader range of biological responses, such as NF- κ B activation, translesion DNA synthesis or cell death. PIDD1 shows features of inteins, a class of self-cleaving proteins, to create different polypeptides from a common precursor protein that allow it to serve these diverse functions. This review summarizes structural information and molecular features as well as recent experimental advances that highlight the potential pathophysiological roles of this unique death fold protein to highlight its drug-target potential.

Death fold-containing proteins in cell death and inflammation

Cell death is an essential part of life in multi-cellular organisms and is critical for development, tissue homeostasis and host defence. Caspases are a class of cysteine-dependent and aspartate-specific endopeptidases that constitute major regulators of cell death and inflammation [1,2]. The activation of caspases can be induced by their dimerization in large signaling complexes such as the apoptosome (Caspase-9), the PIDDosome (Caspase-2) or different types of inflammasomes (CASP1/4/5) [3,4]. Once fully active, these apical caspases are primed to cleave key-substrates that also include but are not limited to distal effector proteins of the same family, such as Caspase-3 or Caspase-7 [5]. The assembly of these platforms is marked by homotypic interactions between conserved structural motifs containing so-called death folds (DF). DFs are six alpha-helical bundle-containing globular domains that form the structural core of death domains (DD), death effector domains (DED), caspase activation and recruitment domains (CARD), and pyrin domains (PYD) [3].

Structural and genomic analysis suggests that many of the networks involved in the innate immune system have evolved from early cell death inducers that contained such DFs [3,6]. During host-pathogen interaction, a plethora of pathways can trigger either cell death, cytokine release or the activation of inflammatory target gene expression [3]. Examples of those are pathogen-associated molecular patterns (PAMPs) converging on inflammasome-driven Caspase-1 activation, which promotes local inflammation through activation of the interleukins IL-1 β and IL-18, as well as Gasdermin D (GSDMD)-mediated pore-formation and pyroptotic cell death [7,8]. Cytochrome c release into the cytoplasm is needed to activate Caspase-9 in the apoptosome, which is still mainly considered anti-inflammatory [9]. Another prominent example that underlines the close relationship between inflammatory signaling and cell death is the signaling cascade downstream of tumor necrosis factor receptor 1 (TNFR1) [10]. Ligand-binding first promotes the formation of the membrane-bound complex I to activate NF- κ B signaling through TRADD, TRAF2 and RIPK1. Sustained signaling leads

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to endocytosis of the multimeric receptor complex and formation of the cytoplasmic complex II which recruits Caspase-8 through FADD to induce apoptosis. Simultaneously, complex II suppresses the inflammatory cell death modality known as necroptosis [3,10]. Finally, the death domain-containing protein PIDD1 [11,12], was long believed to either induce apoptosis by activation of Caspase-2 or cell survival via NEMO-dependent activation of NF- κ B. Yet, selective triggers of PIDDosome activation remained elusive until recently [13].

The p53-induced death domain protein 1 (PIDD1)

Two groups described PIDD1 (*aka*, Leucin-Rich Repeat and Death Domain-Containing Protein, LRDD) as a pro-apoptotic protein in the year 2000 [11,12]. Lin et al. [12] reported that PIDD1 is involved in p53-mediated growth suppression in response to ionizing radiation. Similarly, Telliez et al. [11] identified PIDD1 in a bioinformatics screen for DD interactors of RIPK1. Its pro-apoptotic function in response to disturbances of cellular fitness was assigned to the death domain found at its C-terminal end [11]. Early studies suggested that upon genotoxic stress, PIDD1 kick-starts pro-inflammatory signaling via activation of the NF- κ B pathway, before initiating mitochondrial cell death by activating Caspase-2 [14,15]. However, the fact that PIDD1 knock-out mice failed to show a clear phenotype in response to DNA damage suggested that the pathophysiological context in which PIDD1 becomes relevant was not fully understood [16–18]. Indeed, recent evidence defines PIDD1 as a sensor of centrosome number and regulator of cellular ploidy [19–21].

Transcriptional control of PIDD1 expression

The human *PIDD1* gene is located on chromosome 11p15.5. There are five potential transcript variants of which three have been confirmed to be expressed experimentally [22]. The *PIDD1* gene promoter contains a p53 consensus sequence. In fact, PIDD1 expression is directly regulated by p53 and is strongly induced in response to γ -irradiation [12]. Furthermore, the E2F transcription factor family controls *PIDD1* expression in mouse hepatocytes, and E2F1 overexpression induces *PIDD1* mRNA levels in HeLa cells [23]. In neonatal mouse hepatocytes, the onset of weaning coincides with reduced E2F1/2 activity and increased expression of atypical and repressive E2F7/8, which promote hepatocyte polyploidization by interfering with cytokinesis [24,25]. The switch in transcription factor activity results in decreased *PIDD1* and *CASP2* expression [23]. Whether this regulatory mechanism is also linked to cell cycle progression, during which E2F levels oscillate significantly or if this regulation is specific to hepatocytes is not yet known. Generally, *PIDD1* seems to be expressed weakly in most adult tissues in the mouse, with the highest expression levels in the kidneys, lung and spleen [12]. What controls base line expression of *PIDD1* is currently unknown but protein is also detectable in the absence of p53 [22]. Interestingly, more recent GTEx datasets suggest that *PIDD1*, together with *CASP2*, is most strongly expressed in the brain, skin, small intestine, thyroid and testis, in addition to the aforementioned tissues (Figure 1A). Expression of RAIDD, the adapter protein linking PIDD1 and Caspase-2 via its DD and CARD, respectively, appears to be regulated differently, as its expression levels do not correlate well with the two other genes across tissues [23] (Figure 1B). So far, the PIDD1- and Caspase-2-independent functions of RAIDD have been rarely reported in the literature. Although initially suggested to be involved in TNF signaling [26], a critical role in this inflammatory pathway was not firmly established. However, others have suggested inhibitory roles in NF- κ B signaling downstream of antigen receptors in T cells by interference with the CARMA1 signalosome [27].

PIDD1 structure, protein domain organization and auto-processing

Structurally, PIDD1 is defined by a C-terminal DD that exhibits a typical six alpha-helix bundle comparable to that found in other DD proteins [3,28]. In the years following its discovery, several additional structural features of PIDD1 were defined. On its N-terminus, the protein possesses seven leucine-rich repeats (LRRs). LRRs are often associated with pattern recognition of PAMPs or DAMPs, however, no ligand for PIDD1 has been identified to date. Following the LRRs, PIDD1 contains two ZU-5 domains and a poorly characterized UPA domain (domain found in *UNC5*, *PIDD1* and *ANKYRIN*) [11,29].

To become activated, the full-length precursor of PIDD1, FL-PIDD1, is post-translationally processed at two serine residues, generating an N-terminal fragment (48 kDa PIDD1-N) and two C-terminal fragments (51 kDa PIDD1-C and the 37 kDa PIDD1-CC) [11,30] (Figure 2A). This process depends on a tripeptide motif, His-Phe-Ser, and occurs in an autocatalytic manner, similar to what has been reported for the nuclear pore protein, NUP98 [30,31]. The respective serine — where cleavage occurs — initiates a nucleophilic attack on the preceding scissile amide bond. This process is initiated by the proximity of the side chains in His and Ser.

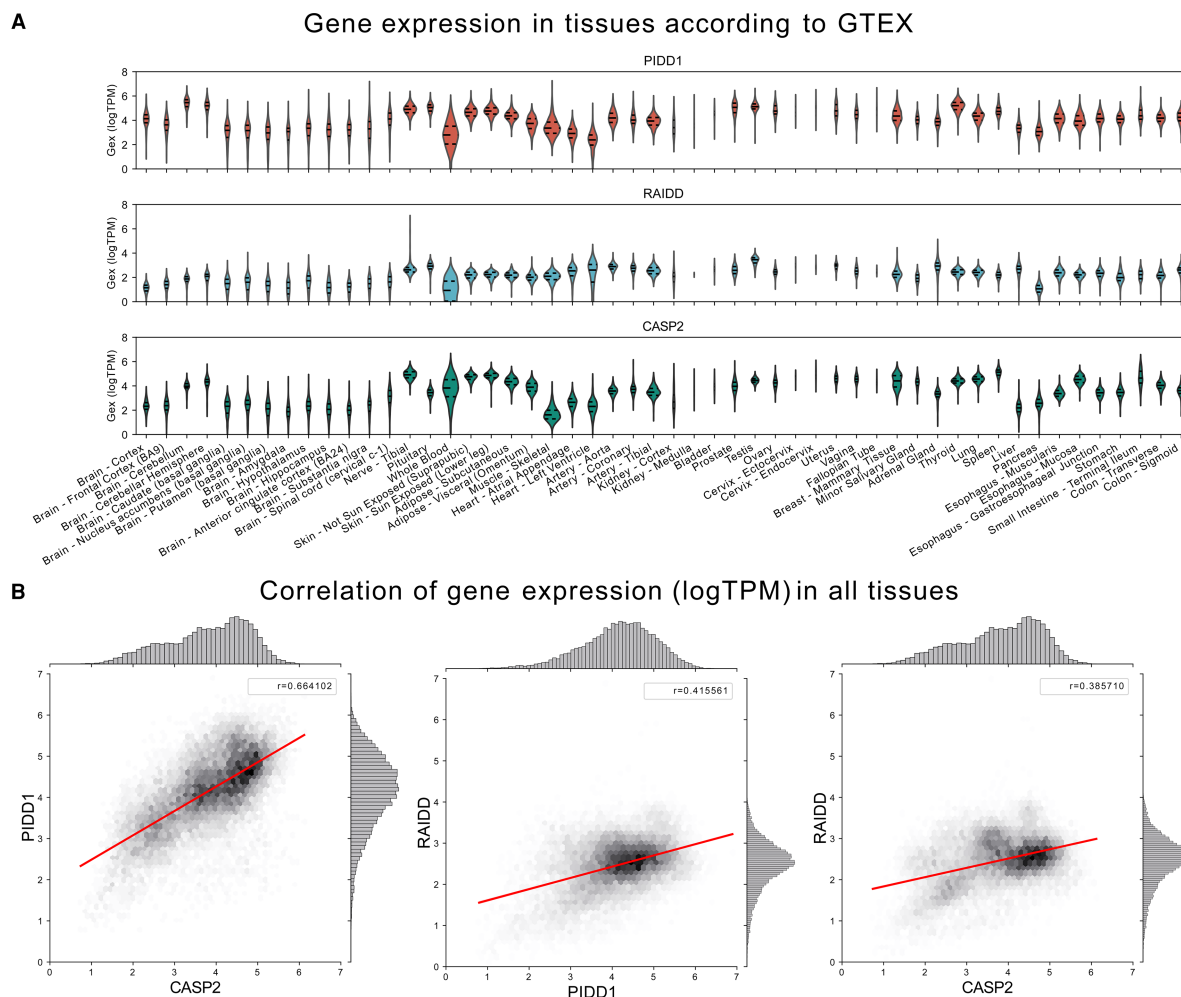


Figure 1. Expression analysis of PIDDosome components, *PIDD1*, *RAIDD*, *CASP2*.

(A) Tissue-specific expression of *H. sapiens* *PIDD1*, *RAIDD* and *CASP2* obtained from the Genotype-Tissue Expression (GTEx v8) project (<https://www.gtexportal.org/>). mRNA expression profiles are shown as log-transformed transcript per million (log (TPM)). (B) Correlation of *PIDD1*, *RAIDD*, and *CASP2* gene expression (logTPM) in human tissues based on data from GTEx v8. Pearson's correlation coefficient shown as *r*-value.

Subsequently, the hydroxyl group induces a reversible N → O acyl shift, forming an intermediate with an ester, replacing the Phe-Ser peptide bond [32]. The ester intermediate is then attacked by a water molecule, and hydrolyzed (Figure 2B). Such autocatalytic processing has also been found in several other proteins with diverse functions such as inteins, Hedgehog, EMR2, N-terminal nucleophile family enzymes (NTNs) or, more recently, two proteins of the NLR-family, NLRP1 and CARD8 [30,33]. In all of these, the side chains of a Ser, Cys or Thr residue act as the nucleophile to initiate self-processing. Interestingly, while the histidine in position -2 in respect to the cleavage site is essential to initiate the second nucleophilic attack in *PIDD1* and *NUP98*, it is exchanged for another serine in *NLRP1* and *CARD8* without impacting auto-processing [34]. Studies on *NUP98* have shown that the conserved tripeptide motif lies within a hydrophobic pocket which is created by the amino acids upstream of the sequence motif. Thus, a longer N-terminal stretch is necessary to allow for cleavage, which may also apply for *PIDD1* [35]. Notably, the N-terminus of *PIDD1* is required for proper processing, as a Δ1–379 deletion mutant is unable to do so efficiently [30].

Lessons from other death fold protein-containing complexes

Structurally, the *PIDD1* C-terminus is closely related to two proteins of the NOD-like receptors (NLR), *NLRP1* and *CARD8*, which both contain a C-terminal FIIND (function to find) domain that resembles the ZU-5 —

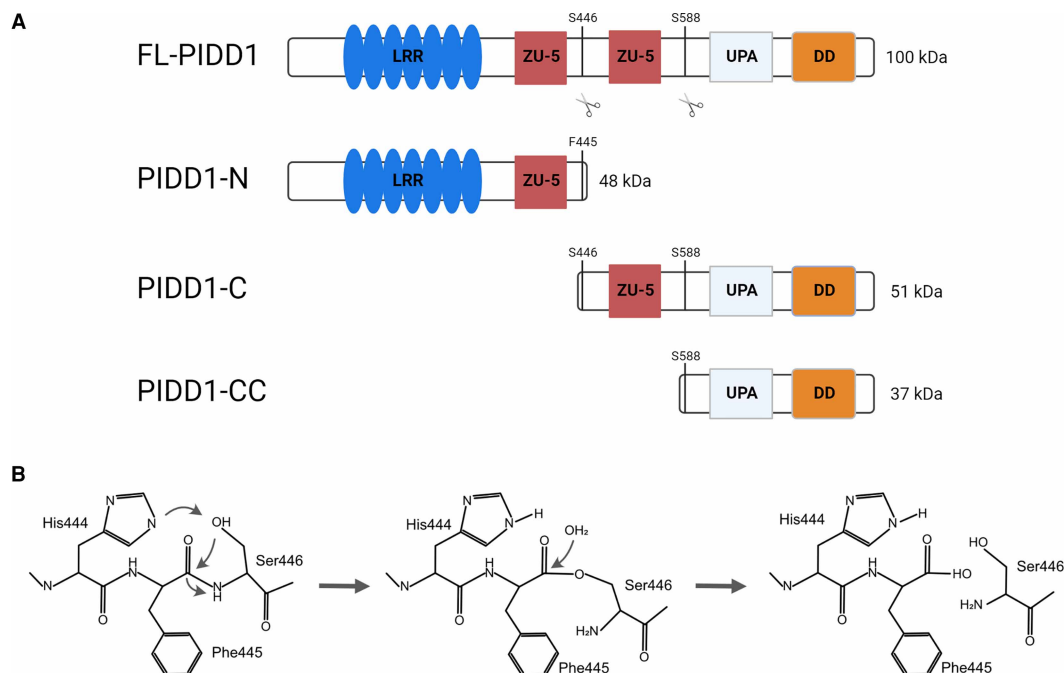


Figure 2. PID1 auto-processing.

(A) Full length PID1 (FL-PID1) is processed into three different fragments. Auto-proteolytic cleavage between F445 and S446 produces PID1-N (48 kDa) and PID1-C (51 kDa). Further cleavage at S588 forms the PID1-CC (37 kDa) fragment. (B) Outline of the chemical transitions needed for PID1 auto-proteolysis. The same mechanism is supposed to occur to generate PID1-C and PID1-CC, in a sequential manner (please see main text for further details).

UPA motif in PID1 [36,37]. NLRP1-mediated Caspase-1 activation depends on homotypic CARD — CARD interaction between the NLRP1 C-terminus and the adapter protein ASC which recruits Caspase-1 into the assembling inflammasome [8,33]. Interestingly, NLRP1, similar to PID1, is constitutively auto-processed in its FIIND domain to release the biologically active C-terminus [37], raising the question how sustained caspase activation is prevented. It seems that the N- and the C-terminal fragments of NLRP1 remain associated after cleavage, thereby sequestering the CARD domain in an inhibitory complex that prevents the initiation of inflammasome assembly [38,39]. Elegant studies have established that triggers of the NLRP1-inflammasome, including the *B. subtilis* toxin Anthrax Lethal Factor and the DPP8/9 inhibitor Val-boro-pro, act by the disruption of this inhibitory complex and subsequent release of the active NLRP1 C-terminus [40,41].

In addition, the domain organization of the PID1 C-terminus is closely related to the UNC5 family of netrin-receptors and to ankyrins such as ANK2. UNC5 proteins are a family of transmembrane receptors which can bind netrin- and netrin-related proteins. Their intracellular domain is composed of a ZU-5 — UPA — DD (ZUD) module, similar to the one found on the PID1 C-terminus. UNC5 receptors are widely expressed during neural development where they are involved in axonal guidance and cell migration [42] and function as dependence receptors with additional roles in apoptosis and tumorigenesis [43]. Interestingly, Wang et al. [29] established that within this ZUD module, the UPA and DD fold back onto the ZU-5 domain, thereby sequestering the DD in an inhibitory state and preventing the induction of apoptosis. ANK2, which contains a similar ZU-5 — ZU-5 — UPA — DD (ZZUD) structure, forms the same supra-module that sequesters its DD [44]. In ANK2, the UPA — DD interact with the first of the two ZU-5 domains, which corresponds to the ZU-5 domain present in PID1-N.

The high similarity of the PID1 C-terminus suggests, that such a mechanism might be relevant for PID1osome activation, especially, since a dominant negative effect of the LRR in PID1-N on NF- κ B activation has been reported [30]. This is further substantiated by co-immunoprecipitation experiments showing a direct interaction between PID1-N and PID1-C [20,30], which coincidentally impairs binding to other interaction partner. Although the mechanism through which the LRR in PID1 exerts its inhibitory effect is

unknown, the features mentioned here are clearly reminiscent of the inhibitory complexes described earlier for NLRP1/CARD8, UNC5 or ANK2.

It therefore appears plausible that a PIDD1-C or -CC molecule is held in an inactive state by binding to the LRR of PIDD1-N until a specific trigger disrupts this interaction. One such trigger might be the phosphorylation of PIDD1 in its DD, which is required for RAIDD binding upon DNA damage [45,46]. However, a specific trigger for PIDDosome formation, e.g. in response to supernumerary centrosomes is still elusive.

PIDD1-containing protein complexes

The most prominent functions of PIDD1 are associated with its ability to nucleate protein complexes through its DD. Initial studies speculated about a host of different DD-containing interaction partners of PIDD1, of which only RAIDD/CRADD and the kinase RIPK1, both carrying a DD, were ultimately confirmed [11,15]. In support of their integration into multi-protein signaling complexes, they co-elute with PIDD1 in high-molecular weight fractions which have been dubbed to correspond to two distinct PIDDosome complexes, namely a complex consisting of PIDD1-CC, RAIDD and Caspase-2 (the ‘Caspase-2-PIDDosome’) and a complex consisting of PIDD1-C, RIPK1 and NEMO/IKK γ (the ‘NEMO-PIDDosome’) [11,13–15]. In addition to these two complexes several PIDD1 interactors have been identified in mass spectrometry analyses, including PCNA [47] and FANCI [46], which engage with PIDD1 in different settings based on other structural features, namely the distal ZU-5 and the distal ZU-5 and UPA domain, respectively (Figure 3). Furthermore, PIDD1 has also been detected in the nucleolus, where it interacts with nucleophosmin 1 (NPM1) via its N-terminal LRRs [48].

The Caspase-2-PIDDosome

In the Caspase-2-PIDDosome five PIDD1-CC molecules interact with seven RAIDD molecules, which in turn recruit the same number of Caspase-2 molecules [28]. Complex formation leads to the proximity-induced

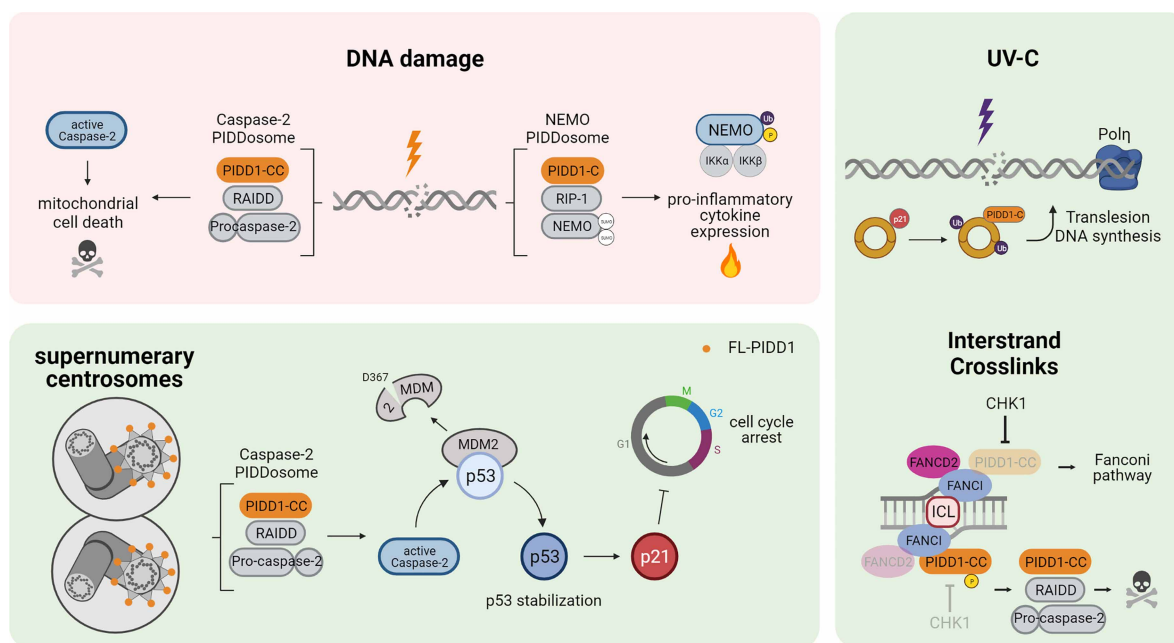


Figure 3. Biological functions of PIDD1-containing protein complexes.

DNA damage is reported to lead to activation of the Caspase-2 – and NEMO – PIDDosome, resulting in either mitochondrial cell death or pro-inflammatory NF- κ B activation and cytokine expression. In response to supernumerary centrosomes, PIDDosome formation leads to p53 stabilization and p21-dependent cell cycle arrest. Under specific circumstances, e.g. after UV irradiation, PIDD1 associates with PCNA to promote Pol η -mediated translesion DNA synthesis. Finally, interstrand DNA crosslinks (ICLs) that cannot be resolved in time trigger the formation of the Caspase-2 – PIDDosome via recruitment of PIDD1-CC to FANCI.

activation of Caspase-2. Careful evaluations of the crystal structure of the DDs of PIDD1 and RAIDD in complex imply an asymmetric assembly mechanism where all of the components have their distinct positions and interaction spectrum. This data also provides a hint to the necessity for PIDD1-CC in the PIDDosome complex since any of the other fragments is likely too bulky to allow complex formation [49–51].

In recent years, our understanding of the mechanisms that trigger PIDDosome assembly has broadened. First, Burigotto et al. [19] have shown that PIDD1 shuttles between the cytoplasm and the mother centriole at the centrosome. It is therefore optimally positioned to respond to alterations in centrosome numbers. Indeed, upon centrosome amplification, e.g. induced by cytokinesis failure or centriole overduplication, the PIDDosome assembles and promotes Caspase-2 activation [21]. The jury is still out as to how and where exactly PIDDosome formation is triggered by supernumerary centrosomes. Two recent studies have shown that knockout of the key factors responsible for the recruitment of PIDD1 to the centrosome, namely the latent sub-distal appendage protein ANKRD26 or the more proximal protein SCLT1, prevent PIDDosome activation in response to cytokinesis failure. This suggests that a priming event needed for PIDDosome assembly happens at the centrosome [19,20]. Notably, processing of PIDD1 into its active fragments is not affected by disrupted centrosomal localization. Furthermore, we were so far unable to find RAIDD or Caspase-2 localizing at the centrosome, indicating that while PIDD1 has to shuttle through the centrosome at some point in order to nucleate the PIDDosome, the centrosome is neither required for PIDD1 processing, nor does it seem to be the location of PIDDosome formation. Of note, PIDDosome activation can promote cell cycle arrest in G1-cells with extra centrosomes but this does not necessarily trigger cell death [21].

Ando et al. [45] observed that forced mitotic entry via CHK1 inhibition in the presence of unresolved DNA damage induces phosphorylation of PIDD1 on T788 in the DD by ATM kinase. This phosphorylation facilitates PIDD1-RAIDD binding and BCL2-independent cell death while simultaneously preventing binding to RIPK1 for cell survival [45,46]. In a follow-up study, Shah et al. described the recruitment of PIDD1 to FANCI, which induces Caspase-2 activation and cell death when interstrand DNA crosslinks (ICLs) cannot be resolved in time by the Fanconi repair pathway [46].

Additional evidence has implicated BUBR1 and other components of the mitotic checkpoint complex (MCC) as negative regulators of Caspase-2-PIDDosome formation [52]. The authors of this study speculate that the mitotic checkpoint complex suppresses a DNA-damage dependent pro-apoptotic program which is in part mediated by the PIDDosome. However, PIDDosome activation is most often evaluated on a bulk of treated cells, as indicated by studies of Ho et al. [53] or Thompson et al. [52], due to technical constraints that prevent the analysis of PIDDosome formation at the single cell level. Therefore, we currently cannot rule out that the PIDDosome-mediated effects on cell death observed in these studies can be traced back to indirect effects of the treatments used, or activation of the PIDDosome at later stages of the cell cycle, e.g. in the next G1 phase. As such, CHK1 inhibition and MCC disruption have both been implicated in mitotic slippage or cytokinesis failure, which might create conditions in which the PIDDosome is activated by the presence of supernumerary centrosomes [54,55]. The current experimental evidence is insufficient to give a final answer whether DNA damage, mitotic arrest and centrosome amplification represent completely separate triggers for PIDDosome activation or whether they are linked. Hence, to shed new light, it would be interesting to see whether ATM- or ATR-dependent PIDD1 phosphorylation is also required for centrosome-mediated PIDDosome activation or likewise whether centrosome localization of PIDD1 presents a requirement for activation downstream of CHK1 inhibition, MCC inactivation or response to DNA interstrand crosslinks.

The NEMO-PIDDosome

It is now established that DNA damage can induce NF- κ B activation and sterile inflammation. In this context, an alternative high-molecular weight complex, consisting of PIDD1, RIPK1 and NEMO, seems to be involved. Upon DNA-damage, exogenous PIDD1-C was shown to translocate to the nucleus and to interact with RIPK1 and NEMO. It is assumed that PIDD1-C and RIPK1 interact via their DDs, based on co-immunoprecipitation experiments performed by Janssens et al. [15]. Surprisingly enough, definite proof that this binding is direct and depends on DD interaction is actually lacking. Complex formation initiates a chain of posttranslational modifications of NEMO, most notably SUMOylation, which lead to the activation of NF- κ B signaling [15,56]. While this NF- κ B activation was initially described as an early pro-survival stimulus to prevent apoptosis immediately after DNA-damage, follow-up studies could not find that the NEMO-PIDDosome mediated a clear survival benefit [57]. Instead, DNA damage-induced NF- κ B activation was shown to trigger pro-inflammatory gene expression in irradiated MEFs that relied in part on RIPK1 and PIDD1 [57].

The recent advances in our understanding of PIDDosome assembly in response to centrosome amplification pose another interesting question: Can extra centrosomes induce the formation of the NEMO-PIDDosome and therefore trigger NF- κ B mediated target gene expression? Such an activation might be relevant primarily in certain myeloid cells that become physiologically polyploid during differentiation and where NF- κ B activation might speed up differentiation or enhance effector function [58–60]. Additionally, we also speculate that NF- κ B activation in cells with aberrantly increased centrosome numbers could trigger an inflammatory response that stimulates their removal by the innate immune system or contribute to the senescence-associated secretory phenotype (SASP), seen in cells with complex karyotypes [61].

Aside from its incorporation into multi-protein complexes mentioned above, a study by Logette et al. [47] showed that PIDD1 interacts with several DNA synthesis and DNA repair factors such as PCNA, RFC4, RFC5 and POL δ in response to UV irradiation. The interaction between PIDD1-C and PCNA is required for dissociation of p21 from PCNA and subsequent PCNA-mono-ubiquitination which leads to recruitment of the translesion DNA synthesis polymerase, POL η , to sites of DNA damage to allow error prone DNA replication, facilitating cell survival [47].

Emerging pathological and physiological roles of PIDD1

PIDD1 as an initiator and effector of p53 function

Early findings about the role of PIDD1 in the p53-response to DNA-damage have been documented in certain human cell lines and zebrafish experiments, they were, however, difficult to reconstruct in primary cell lines derived from PIDDosome mutant mice or universally reproducible across human cell lines. *Pidd1*^{-/-} mouse hematopoietic cells or embryonic fibroblasts do not show decreased apoptosis or increased clonogenic potential upon induction of DNA damage with etoposide or ionizing radiation, even though *in vitro* assays in some human cell lines suggest a direct pro-apoptotic role of the PIDDosome [57,62]. Moreover, *in vivo* evidence of an anti-apoptotic phenotype of *Pidd1*^{-/-} mice comes from skin keratinocytes, which display higher levels of DNA damage after UV irradiation [47].

This prompts the question whether early findings could in fact be partly explained by accidental cellular perturbations that might accompany treatment with DNA damage inducing agents. In line with this, Caspase-2 has been implicated in apoptosis upon treatment with drugs that impair actin filaments or microtubules or promote mitotic errors [53,63,64]. Since the microtubule targeting agents used for the study mentioned above [53] are known to induce mitotic slippage in certain cell types, such as U2OS or A549, we speculate that PIDDosome activation after cytoskeleton disruption may be a result of tetraploidization and the resulting centrosome amplification in a subset of cells. However, the mechanisms that govern whether PIDDosome activation triggers cell death (as observed in the studies of Ho et al. and others [53,63]) or an arrest in G1 phase (as observed by Fava et al. [21]) are currently unknown. Oliver et al. [65] have identified MDM2 as a second bona fide Caspase-2 target, whose cleavage leads to p53 stabilization in the context of Doxorubicin treatment. Yet, p53-induced transcriptional activation of PIDD1 after DNA-damage appears to suffice to allow PIDDosome activation, independent of centrosome amplification and promote secondary Caspase-2-mediated cell death in some settings [66,67].

P53 stabilization does not inevitably induce cell death, but can also contribute to cell cycle arrest and senescence through the transcriptional up-regulation of p21 in certain cell types [68] and subsequently to the secretion of senescence-associated cytokines [69,70]. Accordingly, loss of p53 facilitates survival and cell growth in cells that carry extra centrosomes, leading to CIN and eventually aneuploidy [71,72]. As such, it was tempting to postulate that loss of the PIDDosome should — similarly to loss of p53 — increase CIN and foster aneuploidy. While loss of PIDD1 could indeed facilitate proliferation and growth of cells overexpressing PLK4 to induce centrosome amplification [19,20], our analysis of PIDDosome-defective hepatocytes did not provide evidence for increased levels of aneuploidy in primary hepatocytes or DEN-driven liver cancers [23,73]. This suggests that secondary events that are associated with CIN or aneuploidy, such as DNA damage or proteotoxic stress will eventually activate p53 in a PIDDosome-independent manner to stop the outgrowth of PIDD1-deficient cells.

PIDD1 as a regulator of organogenesis and tissue regeneration

The physiological role of the centrosome-PIDDosome-p53 axis has been explored *in vivo* in naturally polyploid tissues, which have acquired supernumerary centrosomes through different mechanisms [74]. For example,

liver cells become polyploid through a process of incomplete cytokinesis, which is triggered around the time of weaning by changes in insulin signaling [75,76]. Hepatocytes of *Pidd1*, *Raidd* or *Casp2* knockout mice exhibit a substantially higher ploidy compared with wild-type animals [23] (reviewed in Sladky et al. [74,77]). Increased ploidy does not significantly affect liver function in steady state, but is associated with a higher capacity to regenerate liver tissue after hepatectomy [73]. Surprisingly, increased ploidy protects hepatocytes from transformation, and deletion of all three PIDDosome components reduced tumor burden [74].

One interesting aspect of PIDDosome function, which has received some attention in recent years, is associated with the marked expression of Caspase-2-PIDDosome components in the brain. Genetic evidence clearly links perturbations of each of the PIDDosome components to neurodevelopmental or neurodegenerative phenotypes. Loss-of-function mutations in RAIDD and PIDD1 which prevent PIDDosome activation are associated with lissencephaly [78–82], a form of impaired cortical development, and intellectual disability [83,84]. Even though the circumstances which lead to the activation of the PIDDosome in the brain or the cell type where this becomes relevant are not known, the authors speculate that apoptosis induced by the PIDDosome might be critical for proper cortical development [85,86]. Strikingly, however, a number of patients with intellectual disability have been identified that carry mutations in the DD of *PIDD1* which abrogate its ability to activate Caspase-2 [84]. Moreover, the activation of Caspase-2 is implicated in the pathology of Alzheimer's disease (AD) [87,88]. There is evidence that A β -mediated apoptosis is dependent on Caspase-2 and partially also on RAIDD and PIDD1. However, there are also cases where Caspase-2 activation occurs independently of PIDD1, which implies that there might be alternative ways to activate Caspase-2 [48,89].

Open questions

On the one hand, PIDDosome-mediated Caspase-2 activation appears potentially relevant when the DNA repair response is impaired, where it can promote cell death, e.g. upon defective ICL-resolution or prolonged p53 activation [45,46,62,67]. The molecular pathway governing the PIDDosome's response to ICLs has been nicely delineated recently, however, the molecular details concerning execution of Caspase 2--PIDDosome-mediated cell death are still undefined. On the other hand, PIDDosome activation in response to supernumerary centrosomes has put it on the map of cell cycle control, CIN and cancer biology but the relevance of the PIDDosome in tumorigenesis, cancer evolution or response to therapy is far from clear. Moreover, several tissues become polyploid during development and thereby acquire extra centrosomes, which activates the PIDDosome. Whether this is indeed the case in polyploid tissues other than the liver will hopefully be answered in the coming years. Finally, polyploidization is also a feature often observed under inflammatory conditions, most prominently viral infections or bacteria-driven granulomas [90]. There, the NEMO-PIDDosome might be required to coordinate inflammation and immunity in a manner similar to other inflammasomes, and studies addressing these possibilities are eagerly awaited.

Perspectives

- Death fold (DF)-containing proteins are key regulators of cell death and inflammation.
- The DF protein PIDD1 exerts additional effector functions by regulating p53 and cell cycle arrest in response to extra centrosomes.
- Understanding PIDD1 auto-processing will allow selective targeting of its versatile actions to treat diseases associated with deregulated cellular or nuclear ploidy.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Author Contributions

E.S.W. wrote manuscript and prepared figures, T.G.S. provided expression data analysis, I.G.C. edited manuscript, A.V. wrote and edited manuscript and figures.

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Abbreviations

CARD, caspase activation and recruitment domains; DD, death domains; DF, death folds; ICLs, interstrand DNA crosslinks; LRRs, leucine-rich repeats; MCC, mitotic checkpoint complex; PAMPs, pathogen-associated molecular patterns.

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