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ADAR1/ZBP1 in innate immunity, cell death, and disease

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Abstract

ADAR1 and ZBP1 are the only two mammalian proteins that contain a Zα domain, which is thought to bind to nucleic acids in the Z-conformation. These two molecules are crucial in regulating diverse biological processes. While ADAR1-mediated RNA editing supports host survival and development, ZBP1-mediated immune responses provide host defense against infection and disease. Recent studies have expanded our understanding of the functions of ADAR1 and ZBP1 beyond their classical roles and established their fundamental regulation of innate immune responses, including NLRP3 inflammasome activation, inflammation, and cell death, with physiological impacts across development, infectious and inflammatory diseases, and cancer. In this review, we discuss the functions of ADAR1 and ZBP1 in regulating innate immune responses in development and disease.

Keywords

ADAR1; ZBP1; Innate immunity; Inflammation; Cell death; Pyroptosis; Apoptosis; Necroptosis; PANoptosis; PANoptosome; Zα domain; Infection; Development; Cancer; Inflammasome; Caspase; NLRP3; RIPK3; RIPK1; Homeostasis; Infection; Tumorigenesis

The Zα proteins in innate immunity

The innate immune system provides the first line of defense against invading pathogens and homeostatic perturbations. Host cells contain multiple innate immune pattern recognition receptors (PRRs, see Glossary) that sense a wide array of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [1]. Upon PAMP/DAMP recognition, PRRs activate signaling cascades that upregulate the expression of various immune genes, including inflammatory cytokines and chemokines, to direct the immune response [2].

Each PRR responds to distinct PAMPs and DAMPs, and recent studies have greatly advanced our understanding of PRR detection of microbial and aberrant endogenous nucleic

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acids (NAs). PRRs such as Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NLRs), absent in melanoma 2 (AIM2), RIG-I-like receptors (RLRs), in addition to cyclic GMP-AMP synthase (cGAS) can all be involved in sensing NAs in different cellular localizations [3]. NA sensing activates the production of interferons (IFNs), which signal in an autocrine and paracrine manner to initiate downstream Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling to promote the transcription of IFN-stimulated genes (ISGs) and establish an inflammatory, antiviral cellular state [4, 5]. While IFN signaling is essential for infection control, aberrant IFN can cause pathological inflammation. Unlike many other PAMPs, NAs are endogenously produced and highly abundant in the host, posing a risk for self-recognition. Sensing of endogenous RNAs, such as short interspersed nuclear elements and endogenous retroelements (EREs), leads to aberrant IFN production in the absence of infection [6, 7].

To avoid the potential for pathological IFN signaling and to prevent PRR sensing of endogenous double stranded RNA (dsRNA), host RNAs undergo a post-transcriptional base modification catalyzed by enzymes in the Adenosine Deaminase Acting on RNA (ADAR) family [8]. Three ADARs have been identified in mammals, two of which are thought to be active (ADAR1 and ADAR2) [8], while the third (ADAR3) is thought to be catalytically inactive [9] (Box 1, Fig I). ADAR1 is necessary for mouse development and survival [10, 11], and mutations in ADAR1 have been associated with several human autoimmune and autoinflammatory disorders, such as Aicardi-Goutières Syndrome 6 (AGS), systemic lupus erythematosus (SLE), and bilateral striatal necrosis (BSN) [12–14]. These disorders are characterized by aberrant type I IFN production, ISG expression, as well as systemic and chronic inflammation, which can occur due to mutations in ADAR1 in both humans and mice [11, 15, 16]; this links mutations in ADAR1 with the pathophysiology of AGS, SLE, and BSN [12, 15].

One of the key unique structural features of ADAR1 is its Zα domain, which is expected to sense NAs in the Z conformation (Z-NAs) (Box 2, Fig II). The only other molecule that contains a Zα domain in mammals is the Z-DNA binding protein 1 (ZBP1). ZBP1 was initially characterized as an IFN-inducible Z-NA binding protein [17]. Its specific functions remained unclear for many years, and it was suggested to be a cytosolic DNA sensor until the *Zbp1*$^{-/-}$ mouse was generated and found to respond normally to DNA, and to DNA virus infection with normal amounts of IFN production [18]. More insights into ZBP1's functions came with the discovery of its receptor-interacting protein homotypic interaction motif (RHIM) domains that can mediate protein-protein interactions. Specifically, transfection of 293T cells with plasmids expressing ZBP1 and other RHIM-containing proteins showed that ZBP1 interacts with receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and RIPK3 to drive NF-κB and IFN signaling [19]. RHIM-domain dependent interactions between ZBP1 and the M45 protein of murine cytomegalovirus (MCMV) were also reported by transfecting 293T cells; the M45-ZBP1 interaction reduced ZBP1's interaction with RIPK1 or RIPK3 in this overexpression system [20]. Given the role of RIPK proteins in the cell death process called necroptosis [21], as well as observations that i) M45 prevents cell death in transfected NIH3T3 cells treated with TNF or transfected SVEC4–10 cells infected with MCMV; and ii) that M45 inhibits RIPK1 signaling in transfected NIH3T3 cells during MCMV infection [22, 23], a role for ZBP1 in cell death was speculated [20]. Indeed, cell

death in response to MCMV expressing M45 with a mutated RHIM domain ($MCMV^{mM45}$) in NIH3T3 cells transfected with ZBP1 was inhibited upon silencing RIPK3; this indicated that ZBP1 plays a role in necroptosis in this overexpression system [24].

Despite these studies, the endogenous functions of ZBP1 were unclear until ZBP1 was discovered to act as an innate immune sensor regulating NLR family pyrin domain-containing 3 (NLRP3) inflammasome activation and inflammatory innate immune cell death in response to influenza A virus (IAV) infection in mice [25]. Using mouse macrophages deficient in ZBP1 (*Zbp1*$^{-/-}$), this study provided the first genetic evidence for the role of ZBP1 in driving inflammasome activation, as evidenced from caspase-1 activation, IL-1β and IL-18 maturation, as well as activating inflammatory cell death during IAV infection [25]. A follow up study further confirmed the importance of ZBP1 in sensing IAV and regulating inflammatory cell death by showing impaired mixed lineage kinase domain-like (MLKL) and caspase-8 activation during IAV infection in *Zbp1*$^{-/-}$ mouse embryonic fibroblasts (MEFs) [26]. Other studies suggest a role for ZBP1, particularly its Zα domain, in mouse development and survival [7, 27–30].

Beyond ZBP1 and ADAR1's individual functions, a recent discovery showed that there is a regulatory connection between them. Specifically, in murine macrophages, ADAR1 immunoprecipitates with ZBP1 upon stimulation with IFN-β and/or nuclear export inhibitors (NEIs), indicating that ADAR1 interacts with ZBP1; additionally, loss of ADAR1 leads to accelerated ZBP1-dependent cell death in murine macrophages under the same stimulating conditions, suggesting that ADAR1 suppresses ZBP1-mediated inflammatory cell death and can promote homeostasis [29]. Furthermore, this interaction can has been therapeutically modulated to treat melanoma in a murine model [29]. Follow up studies have shown similar connections between ADAR1 and ZBP1 in regulating development, survival, and tumorigenesis in mice [31–34].

In this review, we discuss the biology of ADAR1 and ZBP1, focusing on their roles in regulating cell death and inflammation. We also highlight the role of ADAR1 and ZBP1-modulated inflammatory cell death in development, disease, and tumorigenesis. Moreover, we examine how the interactions between ADAR1 and ZBP1 might influence cancer immunotherapy. Given the broad pathophysiological relevance of these molecules, an improved understanding of their innate immune mechanisms holds promise for the development of putative targeted therapeutic strategies to improve cancer patient outcomes.

ZBP1 in innate immune-mediated inflammatory cell death

Innate immune activation can lead to the induction of cell death to eliminate damaged or infected cells [1]. ADAR1 and ZBP1 are known to modulate regulated cell death (RCD) in several contexts. Among the most well-characterized RCD pathways are pyroptosis, apoptosis, and necroptosis, which are generally modulated in mammalian species by inflammatory caspases and gasdermin family proteins (pyroptosis) [44–46], caspase-3 and −7 (apoptosis) [47, 48], and MLKL (necroptosis) [49, 50].

Although these pathways have historically been defined as segregated and independent processes, mounting evidence shows extensive crosstalk among them [25, 51–55]. Moreover, several sterile insults and infectious agents activate biochemical markers from these three RCD pathways together, and inhibition of a single pathway, such as deletion of gasdermin D (GSDMD) alone, caspase-3 alone, or MLKL alone, is not sufficient to protect against cell death in response to these triggers [56–61]. The observation that cell death is only inhibited in cells deficient in pyroptotic, apoptotic, and necroptotic molecules together, such as *Casp8*$^{-/-}$*Ripk3*$^{-/-}$ or *Casp1*$^{-/-}$*Casp8*$^{-/-}$*Ripk3*$^{-/-}$ bone marrow-derived macrophages (BMDMs) during diverse bacterial, viral, or fungal infections or homeostatic perturbations, highlights that the concept that there may be a unique innate immune inflammatory cell death pathway proposed as PANoptosis; this pathway has been proposed to be regulated by multifaceted PANoptosome complexes that integrate components from other RCD pathways [62]. Decreased incidence of cell death in IAV-infected *Zbp1*$^{-/-}$ BMDMs [25, 63], *Yersinia pseudotuberculosis*-infected *Ripk1*$^{-/-}$ fetal liver-derived macrophages [57], *Francisella novicida*- or herpes simplex virus 1-infected *Aim2*$^{-/-}$ BMDMs [64], or tumor necrosis factor (TNF) plus IFN-γ–stimulated *Casp8*$^{-/-}$*Ripk3*$^{-/-}$ BMDMs [61] establishes ZBP1, RIPK1, AIM2, and caspase-8 as master regulators of PANoptosis.

Sensing of IAV by the ZBP1 Zα2 domain allows ZBP1 to interact with RIPK3 and other associated molecules to form the ZBP1-PANoptosome and induce cell death, as evidenced by reduced cell death in *Zbp1*$^{\Delta Z\alpha 2}$ BMDMs or MEFs in response to IAV infection compared to controls [27, 65], and by co-immunoprecipitation of ZBP1-PANoptosome components ZBP1, NLRP3, RIPK1, caspase-6, caspase-8, and ASC by RIPK3 in transfected HEK293T cells and endogenous BMDMs [63, 66]. The ZBP1-PANoptosome activates the NLRP3 inflammasome to drive IL-1β and IL-18 production in mouse macrophages during IAV infection [25]. However, NLRP3 and caspase-1 are both dispensable for the cell death phenotype, given that ZBP1-dependent activation of apoptotic and necroptotic molecules drives cell death in the absence of inflammasome components. Overall, these findings highlight the key role for ZBP1 in driving NLRP3 inflammasome activation, IL-1β and IL-18 maturation, as well as inflammatory cell death and PANoptosis, in response to IAV infection.

Regulatory connections between ADAR1 and ZBP1 in cell death

Because of similarities between ZBP1 and ADAR1, including their status as ISGs and their similar Zα domains, ADAR1 was hypothesized to mimic ZBP1 as an inducer of cell death. However, *Adar1*$^{-/-}$ murine macrophages undergo accelerated and increased cell death, characterized by robust activation of PANoptotic markers, showing a contrasting phenotype to *Zbp1*$^{-/-}$ murine macrophages that exhibit reduced cell death and less activation of PANoptotic markers [29]. These findings have suggested that ADAR1 might suppress ZBP1-mediated cell death. Indeed, PANoptosis in *Adar1*$^{-/-}$ murine macrophages was inhibited by concurrent deletion of ZBP1 or the Zα2 domain of ZBP1 (*Adar1*$^{-/-}$*Zbp1*$^{-/-}$ or *Adar1*$^{-/-}$*Zbp1*$^{\Delta Z\alpha 2}$ murine macrophages) [29].

Following this initial discovery of a regulatory connection between ADAR1 and ZBP1, several follow up studies subsequently confirmed the ability of ADAR1 to suppress ZBP1-

mediated cell death in several cell types and models [31–34]. For instance, in *Adar1*$^{-/-}$ primary murine fibroblasts, treatment with IFN-α or IFN-γ in vitro in combination with the protein synthesis inhibitor cycloheximide (CHX) induced ZBP1 Zα domain-dependent cell death, characterized by cleavage of caspase-8 and phosphorylation of MLKL [32, 33]. Furthermore, CHX was previously shown to induce robust caspase-1 cleavage and NLRP3 inflammasome activation in murine macrophages [67]; also, the silencing of RHIM-containing proteins RIPK1 and RIPK3, but not toll/interleukin-1 receptor (TIR)-domain-containing adapter-inducing IFN-β (TRIF), resulted in reduced cell death in human HT-29 cells that expressed ZBP1 but had ADAR1^{p150} depleted, indicating that RIPK1 and RIPK3 contributed to ZBP1-mediated cell death induced by ADAR1^{p150} loss [33]. In line with this, the combined deficiency of RIPK3 and caspase-8 (*Ripk3*$^{-/-}$*Casp8*$^{-/-}$) led to impaired ZBP1-dependent PANoptosis induced by NEIs plus IFNs in murine macrophages [29]. Also, the combined pharmacological inhibition of RIPK3 and caspases reduced cell death in *Adar1*$^{-/-}$ fibroblasts [32–34]. Together, these findings suggest that combined loss of multiple RCD effectors is needed to prevent cell death upon ADAR1 loss. Further supporting the important role of the ZBP1 Zα domain in ADAR1-mediated regulation of cell death, lung epithelial type 1 cells, SV40-transformed endothelial cells, and MEFs expressing a mutant form of ZBP1in which the Zα domains were replaced with tandem-inducible dimerization domains derived from the protein FK506, underwent robust cell death when activated using the cell-permeable small molecule B/B; this cell death was blocked in MEFs lacking both caspase-8 and MLKL (*Casp8*$^{-/-}$*Mlkl*$^{-/-}$) or caspase-8 and RIPK3 (*Casp8*$^{-/-}$*Ripk3*$^{-/-}$) [31]. Also, depletion of both isoforms of ADAR1 or specific depletion of the ADAR1^{p150} isoform triggered cell death in human HT-29 cells expressing ZBP1 but not those expressing Zα-domain mutant ZBP1 [33]. These findings suggest that the p150 isoform of ADAR1 is primarily responsible for the inhibition of ZBP1 activation.

Moreover, the ADAR1 Zα domain is required for the editing of RNAs derived from EREs in mammalian cells [68–70], which have been identified as a possible source of dsRNAs that activate ZBP1 [7]. NEI and IFN treatment of wildtype (WT) macrophages or NEI treatment in *Adar1*$^{-/-}$ macrophages leads to the cytosolic accumulation of ERE-derived dsRNA [29], possibly due to altered ADAR1 editing function, although this remains to be fully investigated. Accordingly, NEI treatment sequesters ADAR1 in the nucleus, thereby limiting its ability to carry out its editing function [29]. In addition to these findings in primary mouse macrophages, *Adar1*$^{-/-}$ immortalized macrophages, epithelial cells, and endothelial cells also exhibit increased accumulation of cytosolic dsRNA compared to wildtype cells [34]. Moreover, IFN-α stimulation of murine fibroblasts in vitro increases overall editing of repeats by inducing ADAR1^{p150} expression and editing activity, as evidenced from differential A-to-I editing activity on Alu elements [33]. Stimulation of HT-29 cells transfected to express human ZBP1 with in vitro transcribed Alu-Alu hybrids of the NICN1 and BPNT1 mRNAs induced cell death that was dependent on the Zα domains of ZBP1 [33]. Sequencing of NAs immunoprecipitated by Z22, a monoclonal antibody that detects Z-NAs, identified endogenous Z-NA sources localized in the 3’-untranslated regions of ISGs [34]; this suggested that upregulation of EREs depended on IFN-mediated induction of gene expression.

The suppression of ZBP1-mediated cell death by ADAR1 might be potentially explained by two primary mechanisms: i) competition between ADAR1 and RIPK3 for ZBP1, and ii) competition between ADAR1 and ZBP1 for Z-NAs (Fig 1). Since ADAR1^{p150} and ZBP1 are both ISGs containing Zα domains, it is possible that these proteins interact via their Zα domains, thereby limiting the availability of ZBP1 for binding to RIPK3 and inhibiting cell death, although this remains to be further investigated. Accordingly, ADAR1, but not RIPK3, co-immunoprecipitates with ZBP1 in BMDMs stimulated with IFN, suggesting that IFN stimulation causes endogenous ZBP1 to interact with ADAR1, rather than with RIPK3 [29]; indeed, the ZBP1 Zα2 domain is crucial for this interaction [29, 31]. However, RIPK3 co-immunoprecipitates with ZBP1 in BMDMs stimulated with NEIs or IFN plus NEIs, indicating that sequestration of ADAR1 into the nucleus by NEI treatment or genetic deletion of ADAR1 allows ZBP1 to interact with RIPK3 and subsequently drive cell death [29]. Conversely, genetic deletion of RIPK3 results in an increased interaction between ZBP1 and ADAR1, and the subsequent inhibition of cell death [29]. However, there is also evidence to support an alternative mechanism, namely, that there is competition between ADAR1 and ZBP1 for Z-NAs. Specifically, ADAR1 and ZBP1 are both expected to bind Z-NAs, and chemical crosslinking of proteins to NAs strengthens the interaction between ZBP1 and ADAR1, as evidenced from the higher amount of ZBP1 that co-immunoprecipitates with ADAR1^{p150} in HEK293T cells in response to UV-crosslinking relative to controls [31]. While binding of Z-NAs to the ADAR1 Zα domain prevents Z-NA accumulation in cells, binding of Z-NAs to the ZBP1 Zα domain leads to an interaction between ZBP1 and RIPK3 via RHIM homotypic interactions [29] (Fig 1). Thus, the binding of Z-NAs to ZBP1 Zα domains may also dissociate ZBP1 from the ZBP1-ADAR1 complex.

Overall, ZBP1 interacts with ADAR1 to maintain homeostasis. Loss of ADAR1 triggers Z-NA accumulation and subsequent ZBP1 activation to induce the inflammatory cell death, PANoptosis.

ADAR1 and ZBP1 in tumorigenesis

Dysregulated cell death and inflammatory responses are often associated with tumorigenesis [71]. The Catalogue of Somatic Mutations in Cancer (COSMIC; https://cancer.sanger.ac.uk/cosmic) lists 759 cancer-associated mutations in ADAR1 from 52 histology types such as carcinoma, glioma, and adenoma; 14 are linked to six cancer subtypes in pediatric patients [72] (Fig 2A). Similarly, COSMIC lists 475 cancer-associated mutations in ZBP1 from 43 histology types such as carcinoma, lymphoid neoplasm, melanoma, glioma, and sarcoma; seven are linked to five cancer subtypes in pediatric patients (Fig 2B). Thus, these cancer-associated mutations in ADAR1 and ZBP1 suggest possible roles for ADAR1 and ZBP1 in tumorigenesis.

As discussed above, loss of ADAR1, which can occur as a result of mutations in cancers, leads to the upregulation of ISGs [29]. One such ISG is ZBP1, which can induce PANoptosis and potentially inhibit tumorigenesis. PANoptosis might thus be utilized both naturally and therapeutically [73, 74] to kill cancer cells and suppress tumor growth; for instance, preclinical studies show that PANoptosis induced by TNF and IFN-γ suppresses the growth of transplanted COLO-205 tumors in an NSG mouse model [73]. The combination of TNF

and IFN-γ induces PANoptosis in murine macrophages or cancer cells via the STAT1/IRF1 axis [61, 73]. Loss of IRF1 in myeloid or epithelial cells leads to higher tumor burdens in the colons of mice subjected to the AOM-DSS model of colorectal tumorigenesis relative to controls[73, 74]; this suggests that IRF1 can mediate PANoptosis to prevent tumorigenesis. There are not many studies regarding the role of ZBP1 specifically in tumorigenesis. However, one study showed that a reduced number of tumors in mice deficient in ADAR1 in myeloid cells was reversed by the concomitant loss of ZBP1 or its Zα2 domain (*Adar1*$^{fl/fl}$*LysM*^{cre}*Zbp1*$^{-/-}$ or *Adar1*$^{fl/fl}$*LysM*^{cre}*Zbp1*$^{\Delta Z\alpha 2}$ mice) in the AOM-DSS model of colorectal tumorigenesis [29]. These results suggested a possible tumor-suppressive function in ZBP1 and its Zα2 domain in murine colorectal cancer. The connection between ADAR1 and ZBP1 in regulating tumor growth has been further supported in a melanoma mouse model in which melanoma growth was reduced in *Adar1*$^{fl/fl}$*LysM*^{cre} mice but restored upon concomitant deletion of ZBP1 or its Zα2 domain (*Adar1*$^{fl/fl}$*LysM*^{cre}*Zbp1*$^{-/-}$ or *Adar1*$^{fl/fl}$*LysM*^{cre}*Zbp1*$^{\Delta Z\alpha 2}$ mice) [29]. Furthermore, the implanted melanoma cells were not deficient in ADAR1 or ZBP1 [29], suggesting that tumor extrinsic functions of ADAR1 and ZBP1 might exist to modulate tumorigenesis.

From a therapeutic perspective, we argue that the interaction between ADAR1 and ZBP1 might be considered to ideally improve the efficacy of cancer immunotherapy, pending robust assessment. For example, treatment with NEIs that are specific to chromosomal maintenance 1 (CRM1), also known as exportin-1, have been reported to lead to the nuclear accumulation of ADAR1^{p150} [75] and subsequent cell death in various cell types, including murine macrophages [29, 76]. The combined treatment of NEIs such as KPT-330 or leptomycin B (LMB) with IFNs has sequestered ADAR1^{p150} in the nucleus and activated ZBP1 through dsRNA induction [29]. Moreover, the administration of KPT-330 with IFNs has failed to regress transplanted melanoma in *Zbp1*$^{-/-}$ and *Zbp1*$^{\Delta Z\alpha 2}$ mice, suggesting that PANoptosis that was induced by KPT-330–mediated ADAR1 modulation suppressed tumorigenesis in vivo in a ZBP1- and Zα2 domain-dependent manner [29] (Fig 3). Additionally, a small molecule screen identified CBL0137 as an activator of ZBP1 [34]. In B16-F10 and YUMMER1.7 malignant melanoma mouse models, combined treatment with CBL0137 and anti-PD-1 antibody (immune checkpoint inhibitor), but not either agent alone, regressed tumors in WT but not *Zbp1*$^{-/-}$ mice[34], suggesting a promising role for ZBP1-induced cell death in cancer immunotherapy. Furthermore, compared to ZBP1-deficient MC38 cells, ZBP1-sufficient cells implanted in WT mice have exhibited a growth defect following radiation due to mitochondrial DNA accumulation in irradiated tumor cells; this was accompanied by the production of type I IFNs via the cGAS-STING pathway, as evidenced from *Ifna* mRNA production, formation of STING dimers, and TBK1 phosphorylation [77]. This suggested that tumor cell intrinsic ZBP1 signaling might contribute to certain antitumor effects seen with radiotherapy [77]. In contrast to the presumed tumor suppressive function of ZBP1, MVT-1 cells with *Zbp1* that was genomically deleted using CRISPR-Casp, developed fewer lung metastases compared with MVT-1 cells harboring normal *Zbp1* expression when implanted in FVB/NJ mice; this highlights the possibility that ZBP1 might also bear tumor promoting functions, pending further assessments [78]. We posit that this might likely be due to species or cell type-specific effects, which warrants further investigation. Overall, loss of ADAR1

sensitizes cells to ZBP1-dependent cell death, which appears to be necessary for inhibiting tumorigenesis. Thus, the crosstalk between ADAR1 and ZBP1 might be promising for the development of candidate therapeutic strategies to treat certain cancers – a possibility that certainly merits further attention.

ADAR1 and ZBP1 roles in inflammatory pathology in mice

ADAR1 and ZBP1 have been studied for their roles in development in mice (Box 3, Table 1). Additionally, the role of aberrant ZBP1 function in driving lethality has further been shown in *Adar1*$^{P195A/p150null}$ mice, which carry a mutation within the Zα domain (P195A) on one allele, in combination with deletion of ADAR1^{p150} in the second allele of *Adar1* [84], and in *Adar1*$^{Zα/–}$ mice [32, 70]. Since ADAR1 and ZBP1 likely interact via the ZBP1 Zα domains, and both are expected to bind to a common ligand via their Zα domains, it is possible that aberrant ZBP1 activation might lead to inflammatory pathology and lethality in *Adar1*$^{P195A/p150null}$ and *Adar1*$^{Zα/–}$ mice [31]. Indeed, *Adar1*$^{P195A/p150null}$*Zbp1*$^{–/–}$ mice are phenotypically normal but retain many aspects of the inflammatory signature present in *Adar1*$^{P195A/p150null}$ mice, as evidenced by the presence of pro-inflammatory cytokines and IFNs in *Adar1*$^{P195A/p150null}$*Zbp1*$^{–/–}$ mice [31]; this may be due to aberrant activation of melanoma differentiation-associated protein 5 (MDA5) driving IFN-dependent inflammation, although this remains to be assessed. However, not all *Adar1*$^{Zα/–}$*Zbp1*$^{–/–}$ and *Adar1*$^{Zα/–}$*Zbp1*$^{Zαnull/Zαnull}$ mice survive to adulthood, while *Adar1*$^{Zα/–}$*Mavs*$^{–/–}$ mice are completely rescued from lethality [32, 33]; this suggests that both ZBP1-dependent and -independent roles of the MDA5–mitochondrial antiviral-signaling protein (MAVS) signaling axis might contribute to the observed pathology in *Adar1*$^{Zα/–}$ mice. Moreover, there is increased survival in *Adar1*$^{Zα/–}$*Zbp1*$^{–/–}$ mice compared to *Adar1*$^{Zα/–}$*Zbp1*$^{Zαnull/Zαnull}$ mice, suggesting that ZBP1 might exert Zα domain-independent functions [32]. ZBP1 deficiency markedly suppresses the type I IFN signature, though to a lesser extent than MAVS deficiency does in *Adar1*$^{Zα/–}$ mice [32]; this is evidenced by the combined loss of ZBP1 and MAVS in *Adar1*$^{Zα/–}$ mice completely normalizing the expression of ISGs to the expression seen in *Adar1*$^{Zα/WT}$ mice, while this is not the case in *Adar1*$^{Zα/–}$*Mavs*$^{–/–}$ mice [32]. These findings suggest that ZBP1 might contribute to the ISG response independently from MAVS. In fact, about 40% of *Adar1*$^{–/–}$*Mavs*$^{–/–}$*Zbp1*$^{–/–}$ mice survive to adulthood, in contrast to *Adar1*$^{–/–}$*Mavs*$^{–/–}$ mice which die shortly after birth; this suggests that ZBP1 and MAVS signaling together may drive lethality in ADAR1-deficient mice [32]. Altogether, MAVS-dependent and MAVS-independent ZBP1 functions may drive lethality in mice lacking ADAR1.

ZBP1-mediated inflammatory cell death also potentially contributes to the lethality of *Adar1*$^{–/–}$*Mavs*$^{–/–}$, *Adar1*$^{P195A/p150null}$, or *Adar1*$^{Zα/–}$ mice. Indeed, loss of another molecule that is crucial for ZBP1-mediated cell death, RIPK3, in *Adar1*$^{–/–}$*Mavs*$^{–/–}$ mimics the survival rate of *Adar1*$^{–/–}$*Mavs*$^{–/–}$*Zbp1*$^{–/–}$ mice [32]. Moreover, ablation of RIPK3 partially extends the survival of *Adar1*$^{P195A/p150null}$ mice, although this fails to fully reverse lethality [31], suggesting that RIPK3-independent functions in ZBP1 might also contribute to inflammatory pathology in *Adar1*$^{P195A/p150null}$ mice. Furthermore, deletion of MLKL fails to rescue the lethality of *Adar1*$^{P195A/p150null}$ mice [31], suggesting that blocking necroptosis alone might not be sufficient to limit the function of ZBP1 in these mice. Moreover, loss

of B-cell lymphoma 2 (Bcl2) antagonist/killer 1 (BAK) and Bcl2-associated X protein (BAX) (*Bak*$^{-/-}$*Bax*$^{-/-}$) fails to prevent lethality in *Adar1*$^{E861A/E861A}$ mice [85], implying that loss of apoptosis alone may not be sufficient for protection either. Considering the involvement of ZBP1 in PANoptosis, loss of apoptotic or necroptotic molecules alone might not be sufficient to block the pathology in ADAR1-deficient mice, and this is relevant because loss of ADAR1 triggers ZBP1-dependent activation of PANoptosis [29]. Moreover, combined deletion of MLKL and caspase-8 in *Adar1*$^{P195A/p150null}$ or *Adar*1$^{Zα/–}$ mice (*Adar1*$^{P195A/p150null}$*Casp8*$^{-/-}$*Mlkl*$^{-/-}$ or *Adar*1$^{Zα/–}$*Casp8*$^{-/-}$*Mlkl*$^{-/-}$ mice) still fails to rescue the lethality of *Adar1*$^{P195A/p150null}$ or *Adar*1$^{Zα/–}$ mice [31, 70], suggesting that ZBP1-mediated functions might still contribute to lethality in the absence of apoptotic and necroptotic molecules in *Adar1*$^{P195A/p150null}$ or *Adar1*$^{Zα/–}$ mice. This might be due to the formation of the ZBP1-PANoptosome and induction of PANoptosis, though extensive work is required to confirm this.

Molecularly, the role of ZBP1 in the ADAR1-deficiency–mediated pathology might be explained through the accumulation of specific endogenous NAs. ERE expression is increased in the spleen of *Adar1*$^{Zα/–}$ mouse pups compared with *Adar1*$^{Zα/WT}$ pups, suggesting that ADAR1 suppresses ERE production [32]. ZBP1 deficiency in *Adar1*$^{Zα/–}$ mice inhibits the upregulation of ERE expression, possibly due to suppressed IFN production in *Adar1*$^{Zα/–}$*Zbp1*$^{-/-}$ mice, suggesting that IFN is required for the upregulation of ERE [32].

Altogether, ADAR1 and ZBP1, and their interactions, are strongly implicated in mouse ontogeny, and their dysfunction may contribute to inflammation. Inflammatory cell death, and particularly ZBP1-mediated PANoptosis, can contribute to the pathology caused by impaired ADAR1 function, and the biochemical features of this cell death can be influenced by cell and tissue types, depending on the expression of the pathway components and regulatory proteins. Alternatively, ZBP1-mediated PANoptosis may indirectly contribute to inflammatory responses through the release of DAMPs from dying cells and subsequent activation of other immune sensors. Overall, evidence suggests that blocking ZBP1-mediated PANoptosis might mitigate pathology driven by alterations in ADAR1, while inducing PANoptosis by disrupting the inhibitory interaction of ADAR1-ZBP1 might be beneficial to help prevent tumorigenesis.

Concluding remarks

ADAR1 and ZBP1 can play key roles across innate immunity, inflammatory cell death, homeostasis, development, autoinflammatory diseases, host defense, and tumorigenesis. Loss of ADAR1 stimulates a global IFN response due to sensing of self dsRNA by the MDA5-MAVS axis, culminating in IFN production and ISG upregulation. Consequently, embryonic lethality caused by ADAR1 deficiency in mice is partially rescued by concomitant deletion of MDA5 or MAVS. Moreover, combined deletion of ZBP1 and MAVS in *Adar1*$^{-/-}$ mice further improves their survival, suggesting that synergism of ZBP1 and MAVS signaling might drive lethality in ADAR1-deficient mice [32]. In humans, ADAR1 loss of function or MDA5 gain of function mutations have been identified in rare autoimmune diseases such as AGS [12, 86]. Furthermore, mutations in the ADAR1 Zα

domain cause AGS and BSN when combined with alleles that cause loss of ADAR1^{p150} expression [32]. These conditions are mimicked in *Adar1*$^{mZα/–}$ or *Adar1*$^{P195A/p150null}$ mice, and these phenotypes are rescued by concomitant deletion of ZBP1 or the ZBP1 Zα domains, establishing a possible detrimental role of ZBP1 in some inflammatory diseases such as sepsis, skin inflammation, and bowel diseases [30, 87, 88].

ADAR1 and ZBP1 are also implicated in tumorigenesis. Increased ADAR1 expression or activity leads to cancer development through its suppression of IFN production and anti-tumor responses in mice [89, 90]. Therefore, ADAR1-mediated inhibition of the endogenous dsRNA sensing mechanism might serve as a putative checkpoint. The upregulation of ZBP1 induced by loss of ADAR1 triggers inflammatory cell death which might be beneficial in suppressing tumorigenesis, but detrimental in inflammatory diseases such skin and bowel diseases (see Outstanding Questions). Preclinical strategies to inhibit the regulatory interaction between ADAR1 and ZBP1 might include approaches such as using NEI treatment to restrict ADAR1 to the nucleus in combination with a Z-NA–inducing small molecule such as curaxin to activate ZBP1; some of these have been effective in inhibiting melanoma growth in mice xenograft models [29, 34] and merit further attention. The studies discussed in this review have primarily used mouse models to understand the interplay between ADAR1 and ZBP1 in innate immunity, PANoptosis, and disease outcomes. There are limitations in the extrapolations we can make from mouse models. However, given their key functions in health and disease, we argue that future studies on ADAR1 and ZBP1 can be important in identifying the full spectrum of disease processes in which these two proteins, and their regulatory relationship, are implicated.

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Glossary

Aicardi-Goutières Syndrome 6
rare genetic inflammatory disorder affecting the brain, spinal cord, skin, and immune system; characterized by elevated expression of IFNs. AGS is caused by biallelic variants in *ADAR1*

AOM-DSS
Azoxymethane (AOM) is a metabolite of dimethylhydrazine that acts as a procarcinogen, requiring metabolic activation to form DNA-reactive products. Dextran sulfate sodium (DSS) is a synthetic sulfated polysaccharide composed of dextran. AOM-DSS is a widely used combination for the induction of colitis-associated colorectal tumorigenesis in rodents

Apoptosis

non-lytic form of programmed regulated cell death; generally immunogenically silent. It involves the activation of initiator caspases, e.g. caspase-8 and -9, and executioner caspases, e.g. caspase-3 and -7

Bilateral striatal necrosis
group of neurodegenerative diseases characterized by symmetrical degeneration of the putamen, caudate nucleus, and globus pallidus

Damage-associated molecular pattern
Endogenous molecules that are released by or found on damaged or dying cells that activate the innate immune system by interacting with PRRs

Deaminase domain (or adenosine deaminase domain)
portion of a protein that encodes a purine metabolism enzyme that catalyzes the irreversible conversion of adenosine and deoxyadenosine to inosine and dexoyinosine, respectively

Endogenous retroelements
DNA sequences which can duplicate and move to new locations in the genome. They constitute nearly 50% of the human genome

Immunotherapy
biological therapy for the treatment of certain diseases, it may involve approaches such as monoclonal antibodies to modulate the host immune system

KPT-330
orally bioavailable selective inhibitor of nuclear export which irreversibly binds to and inhibits the function of CRM1, or XPO1. KPT-330 (Selinexor) is FDA approved for the treatment of multiple myeloma and diffuse large B cell lymphoma

Necroptosis
inflammatory regulated cell death pathway occurring in response to caspase-8 inhibition; involves activation of RIPK3 and MLKL

Nuclear export sequence
short peptide containing hydrophobic residues in a protein that targets it for export from the cell nucleus to the cytoplasm using nuclear transport. The NES is recognized and bound by exportins

PANoptosis
innate immune inflammatory cell death pathway regulated by PANoptosome complexes which assemble in response to innate immune sensing of pathogens, PAMPs, DAMPs, or other molecular components (i.e., cytokines)

PANoptosome
multiprotein complex which assembles in response to innate immune stimuli and integrates components from other cell death pathways (such as caspase-1 from pyroptosis, caspase-8 from apoptosis, and RIPK3 from necroptosis) to execute PANoptosis

Pattern recognition receptors
Germline-encoded molecules that can directly recognize the specific molecular structures from pathogens or damaged cells

Pathogen-associated molecular pattern
Small molecular motifs conserved within a class of microbes that are recognized by PRRs

Pyroptosis
inflammatory regulated cell death associated with inflammasome formation, caspase-1 activation, and execution by gasdermin family members. It is essential for the maturation of IL-1β and IL-18

Regulated cell death
controlled form of cell death that follows a prescribed pathway in response to a triggering stimulus

Z-nucleic acids
Left-handed double helix nucleic acid structures of Z-DNA and Z-RNA in which the helix winds to the left in a zigzag pattern

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Box 1.

Domain characterization of ADAR and ZBP1

ADARs are highly conserved across vertebrates. The three human ADARs are composed of similar domain arrangements. Starting from the C-terminus, each ADAR has a catalytic deaminase domain, followed by several dsRNA binding domains (dsRBDs) (Fig I, Box 1). ADAR1 contains three dsRBDs, while ADAR2 and ADAR3 contain two. ADAR1's third dsRBD contains a nuclear localization sequence (NLS). There are two isoforms of ADAR1, ADAR1^{p110} and ADAR1^{p150} [91–93]. While both isoforms contain a Zβ domain, the p150 contains the Zα domain, which has a nuclear export sequence (NES). Due to the presence of both the NES and the NLS, ADAR1^{p150} shuttles between the nucleus and cytoplasm. ADAR1^{p110} and ADAR2 are predominantly localized in the nucleus due to their NLS [36, 75, 94]. ADAR1^{p110} is constitutively expressed, but ADAR1^{p150} is induced by IFNs [93, 95]. While ADAR1 and ADAR2 are catalytically active and expressed in many tissues, ADAR3 expression is restricted to the brain, and it has no catalytic activity. Point mutations at a key glutamate residue, E912A (ADAR1) and E396A (ADAR2), are sufficient to inactivate ADAR1 and ADAR2, respectively [96]. Loss of ADAR1 leads to embryonic lethality in mice [10, 11]. Similar to ADAR1 deficiency, *Adar2*$^{-/-}$ mice normally die within 3 weeks of birth, but this early postnatal lethality is rescued when the ADAR2 editing site in the *Gria2* transcript, which encodes the main subunit of glutamate receptor 2, is modified to constitutively express an arginine instead of a glutamine at the Q/R site [97, 98]. This suggests that this transcript is the most physiologically important substrate of ADAR2. Knockdown of ADAR2 leads to upregulation of several genes associated with inflammation and immunity. Moreover, ADAR2 is important for viral recognition and replication [99]. *Adar3*$^{-/-}$ mice are grossly normal but display cognitive defects in learning and memory, and global A-to-I editing is essentially unchanged [100]. Biochemical and molecular analysis have indicated that ADAR3-expressing U87-MG glioblastoma cells exhibit increased nuclear localization of NF-κB and production of pro-inflammatory cytokines, suggesting that ADAR3 promotes NF-κB activation [101].

ZBP1, initially named DLM-1 and then DAI (DNA-dependent activator of IFN-regulatory factors), contains two N-terminal Zα domains, Zα1 and Zα2, followed by two receptor-interacting protein homotypic interaction motif (RHIM) domains and an undefined C-terminal domain (Fig II, Box 2). Like *ADAR1*, *ZBP1* is an IFN-inducible gene, and both are induced during various infections [25, 29]. ZBP1 is primarily cytoplasmic; however, it can form nuclear foci in activated cells [102, 103].

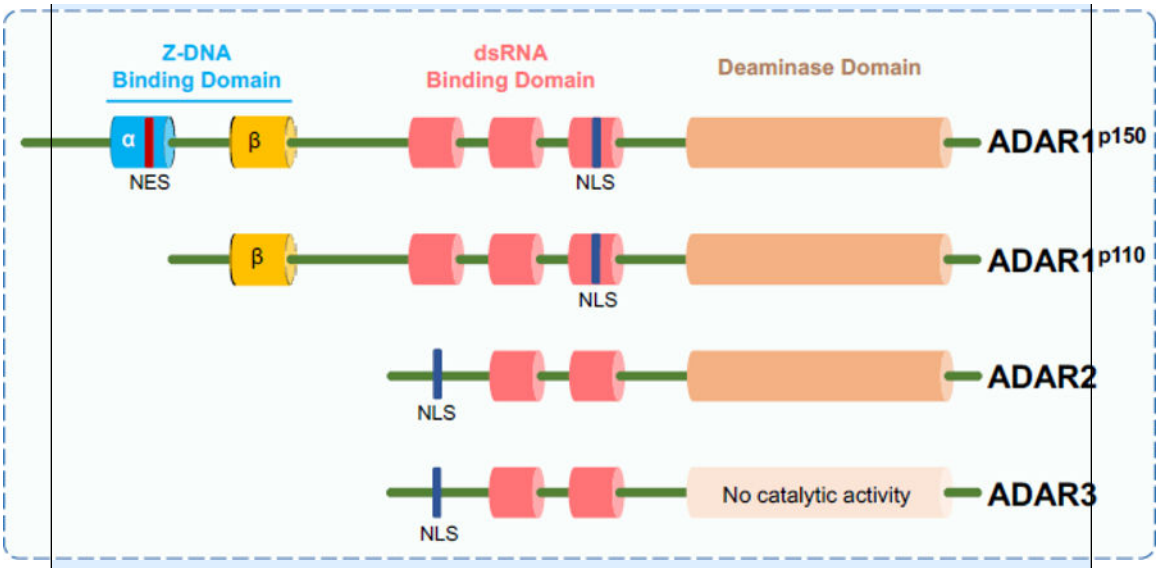

Figure I in Box 1: Organization of domains in human ADAR family proteins

ADAR1, ADAR2, and ADAR3 are three members of the human ADAR family of proteins. All three proteins contain a dsRNA binding domain and a deaminase domain. While the deaminase domains of ADAR1 and ADAR2 are active, the deaminase domain of ADAR3 lacks catalytic activity. Alternative promoters and splicing give rise to two forms of ADAR1, the interferon (IFN)-inducible ADAR1^{p150} isoform and the ADAR1^{p110} isoform. The N-terminal region of the p150 isoform possesses two Z-DNA binding domains (Zα and Zβ). While ADAR1^{p150} contains both NES and NLS signals, all other ADAR members contain only an NLS signal. ADAR, adenosine deaminase acting on RNA; dsRNA, double-stranded RNA; NES, nuclear export signal; NLS, nuclear localization sequence.

Box 2.

Zα domains across species and mechanisms of binding

Since the discovery of ADAR1, there have been ongoing efforts to identify other Zα domain-containing proteins. Zα domains have been reported in various non-mammalian proteins including Protein Kinase Containing Z-DNA binding domains (PKZ) in fish [104]; E3L in poxvirus family members, such as vaccinia virus, Yaba-like disease virus, and orf virus [105]; ORF112 in fish herpesviruses, such as Cyprinid herpesvirus 3 (CyHV3) [106, 107]; and RBP7910 in *Trypanosoma brucei*, a human-fly parasite that can cause African Trypanosomiasis or sleeping sickness [108] (Fig II).

Zα domains are expected to bind NAs in the Z conformation. NAs naturally exist in three major forms: a compact right-handed A form, a loose right-handed B form, and the unique left-handed Z form. Unlike the anti-conformation base arrangement in the A and B forms, the nucleoside bases in the Z form adopt alternating syn- and anti-conformation bases, assigning a distinctive left-handed double helical structure with a zigzag backbone [35]. Almost two decades after the discovery of the Zα domain of ADAR1, the physiological relevance of Z-nucleic acids (Z-NAs) has been experimentally difficult to confirm due to technical limitations of detecting Z-NAs. Unbound Z-NAs are energetically unstable, as they are in a higher energy configuration [36], which allows the amino acids in the binding pocket of the Zα domain to interact with the zigzag sugar-phosphate backbone of Z-NAs [37, 38]. Three conserved residues, N173 and Y177 in the α helix and W195 in the β sheet of the human ADAR1 Zα domain, play central roles in the interaction with Z-NAs [39, 40], and a W195A substitution causes a complete loss of binding to Z-NAs [41].

The amino acid sequence of the ZBP1 Zα1 domain is approximately 35% identical to that of the Zα domain of ADAR1 [42], with a slightly lower conservation in the ZBP1 Zα2 domain. The interaction between the ZBP1 Zα domains and Z-NAs is mediated by three conserved core residues (Y145, N141, and W162). However, aside from these residues, other interactions with Z-NAs seem to be different between ZBP1 and ADAR1 [43].

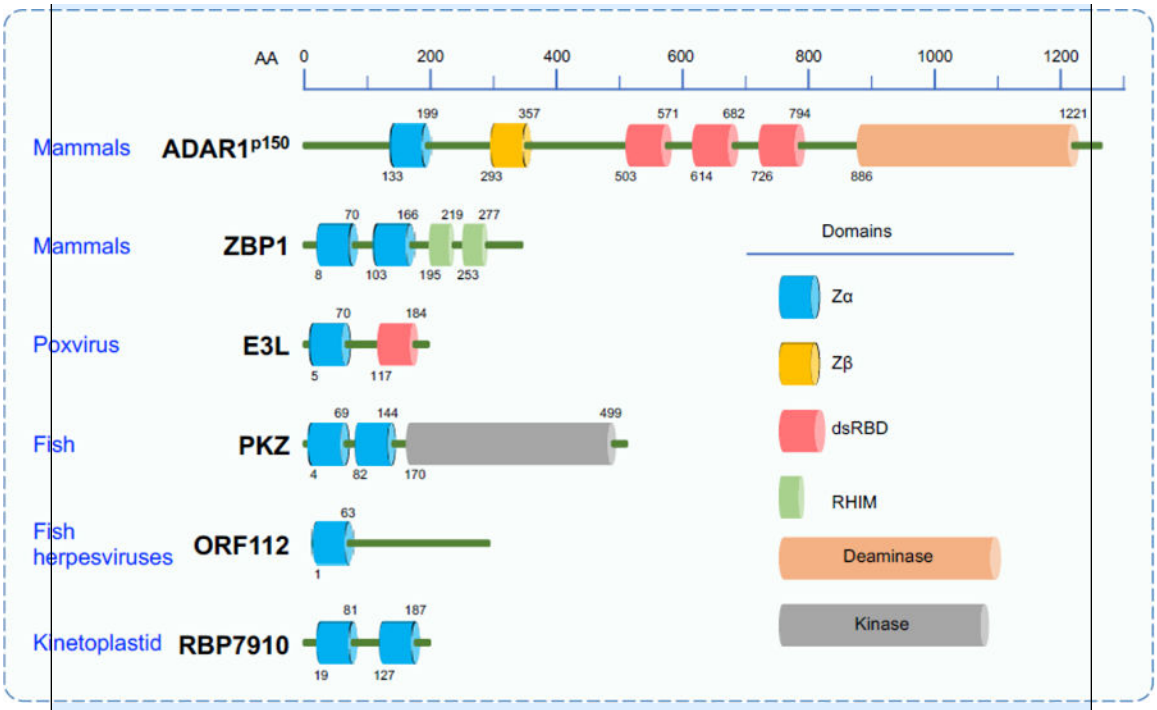

Figure II in Box 2: Domain organization of Zα-containing proteins

ADAR1^{p150}, ZBP1, E3L, PKZ ORF112, and RBP7910 are Zα domain-containing proteins. While ADAR1^{p150}, E3L, and ORF112 contain a single Zα domain, ZBP1, PKZ, and RBP7910 contain two Zα domains. ADAR1 and E3L also possess dsRNA binding domains. ZBP1 contains two RHIM domains. The species where each protein can be found is designated in blue. AA, amino acid; ADAR1, adenosine deaminase acting on RNA 1; dsRBD, double-stranded RNA binding domain; dsRNA, double-stranded RNA; PKZ, protein kinase containing Z-DNA binding domains; RHIM, receptor-interacting protein homotypic interaction motif; ZBP1, Z-DNA binding protein 1.

Box 3.

ADAR1 and ZBP1 roles in mouse development

Both ADAR1 and ZBP1 play crucial roles in mouse development and survival (Table 1). *Adar1*$^{-/-}$, *Adar1*$^{p150null/p150null}$ (*Adar1*^{p150null}), and *Adar1*$^{E861A/E861A}$ mice, which have a point mutation in the ADAR1 catalytic domain, are all embryonically lethal. This lethality is accompanied by hyperproduction of type I IFNs, upregulation of ISGs, and widespread cell death, particularly in liver hematopoietic cells [10, 11, 79].

MDA5 and the downstream adaptor protein MAVS have been relatively well characterized in the ADAR1 pathway. The concurrent deletion of the cytoplasmic sensor MDA5 or the downstream adaptor protein MAVS rescues the embryonic lethality of *Adar1*$^{-/-}$ and *Adar1*^{p150null} mice, though these mice still undergo lethality shortly after birth, which allows the normal lifespan of *Adar1*$^{E861A/E861A}$ mice [33, 80–82]. These results suggest that overt PRR activation and the resulting innate immune responses may be responsible for the lethality and hyperactivated IFN signaling observed in these mice.

Evidence also suggests that loss of ADAR1 leads to upregulation and activation of other innate immune sensors. For instance, despite the rescue of embryonic lethality in *Adar1*$^{-/-}$ and *Adar1*^{p150null} mice by deletion of MDA5 or MAVS (*Adar1*$^{-/-}$*Mda5*$^{-/-}$, *Adar1*$^{-/-}$ *Mavs*$^{-/-}$ or *Adar1*^{p150null}*Mda5*$^{-/-}$, *Adar1*^{p150null}*Mavs*$^{-/-}$), these mice still die shortly after birth [33, 80–82]. Moreover, loss of the type I IFN receptor (*Ifnar1*$^{-/-}$) or combined loss of the type I and II receptors (*Ifnar1*$^{-/-}$*Ifngr*$^{-/-}$) in *Adar1*$^{-/-}$ mice (*Adar1*$^{-/-}$ *Ifnar1*$^{-/-}$ or *Adar1*$^{-/-}$ *Ifnar1*$^{-/-}$*Ifngr*$^{-/-}$) fails to mimic the survival observed in *Adar1*$^{-/-}$ *Mda5*$^{-/-}$ or *Adar1*$^{-/-}$*Mavs*$^{-/-}$ mice [81, 83]. Type I IFN production is markedly reduced in *Adar1*$^{-/-}$*Mda5*$^{-/-}$ mice, but the upregulation of ZBP1 is only modestly affected [33]. Therefore, ZBP1 may contribute to the postnatal lethality in *Adar1*$^{-/-}$*Mavs*$^{-/-}$ and *Adar1*^{p150null}*Mavs*$^{-/-}$ mice [31–33]. Indeed, survival is improved in *Adar1*$^{-/-}$*Mavs*$^{-/-}$ mice expressing a mutant form of ZBP1 with impaired binding to Z-NAs (*Adar1*$^{-/-}$ *Mavs*$^{-/-}$*Zbp1*$^{mZα/mZα}$), and in *Adar1*$^{-/-}$ *Mavs*$^{-/-}$*Zbp1*$^{-/-}$ or *Adar1*^{p150null}*Mavs*$^{-/-}$*Zbp1*$^{-/-}$ mice [31–33]. However, deletion of ZBP1 alone is not sufficient to allow *Adar1*$^{-/-}$ or *Adar1*^{p150null} mice to survive to birth [31, 32], suggesting that developmental lethality is mediated by the simultaneous activation of ZBP1, MDA5, and quite likely, other pathways.

Outstanding questions

- How does insertion of inosine into dsRNAs block MDA5 sensing in mammalian cells?
- Can ZBP1 sense this modified dsRNA in mammalian cells? If not, does A-to-I editing prevent dsRNAs from attaining the Z-conformation?
- ZBP1 contains a Zα1 and a Zα2 domain in mammalian cells. Which Zα domain of ZBP1 interacts with ADAR1?
- ZBP1 also has two RHIM domains; however, only the RHIM domain closest to the C-terminus mediates the interaction between ZBP1 and RIPK1, RIPK3, or TRIF in mammalian cells, contributing to inflammatory responses or cell death. What is the function of having multiple RHIM domains in ZBP1?
- Caspase-6 promotes the interaction between RIPK3 and ZBP1 that drives cell death during IAV infection in murine macrophages. Does caspase-6 influence the interaction between ADAR1 and ZBP1?
- Since both ZBP1 and ADAR1 bind to nucleic acids (NA) in mammalian cells, do ZBP1 and ADAR1 compete for NAs?

Highlights

- ADAR1 and ZBP1 are mammalian interferon-inducible proteins containing Zα domains that regulate innate immune responses across development and disease.
- Mutations in ADAR1 are associated with several autoimmune and autoinflammatory disorders in humans, such as Aicardi-Goutières Syndrome 6, systemic lupus erythematosus, and bilateral striatal necrosis.
- There is a regulatory relationship between ADAR1 and ZBP1 in mammalian cells, where ADAR1 suppresses ZBP1-mediated inflammatory cell death, PANoptosis.
- ZBP1-mediated PANoptosis contributes to the pathology caused by impaired ADAR1 function in mice.
- Using nuclear export inhibitors to disrupt the interaction between ADAR1 and ZBP1 by sequestering ADAR1 in the nucleus, and activating ZBP1 through the induction of dsRNA, have shown promise in preclinical mouse models.

Significance

ADAR1 and ZBP1 are the only two mammalian Zα-containing proteins; they regulate innate immune responses crucial for survival, development, and host defense against infection, inflammatory diseases, and cancers. While the interaction of ADAR1 with ZBP1 suppresses cell death, the interaction of RIPK3 with ZBP1 drives innate immune inflammatory cell death (i.e. PANoptosis). Molecules that can modulate the ADAR1-ZBP1 or RIPK3-ZBP1 interaction have therapeutic potential for the treatment of inflammatory and infectious diseases.

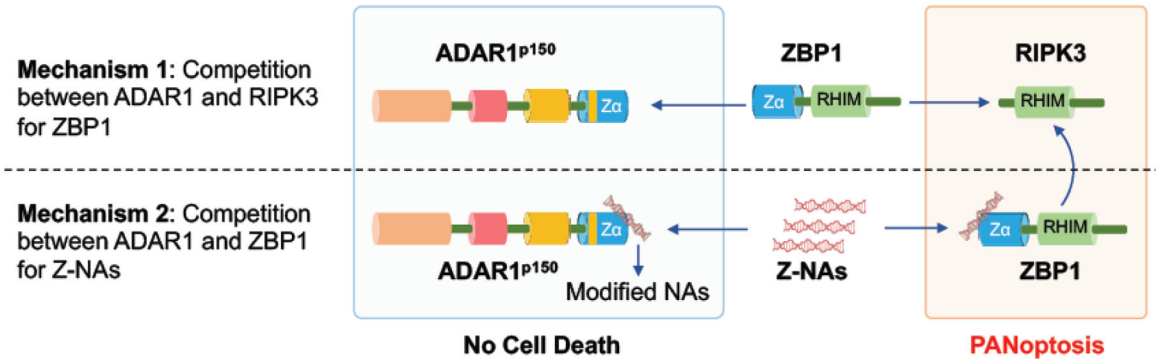

Figure 1: Possible mechanisms of ADAR1 suppression of ZBP1-mediated PANoptosis

Two plausible mechanisms of ADAR1 regulation of ZBP1-mediated PANoptosis in mammalian cells may exist: 1) competition between ADAR1^{p150} and RIPK3 for ZBP1 or 2) competition between ADAR1^{p150} and ZBP1 for Z-NAs. ZBP1 has both Zα and RHIM domains. With its Zα domains, ZBP1 interacts with the Zα domain of ADAR1^{p150}; with its RHIM domains, ZBP1 interacts with RIPK3. Competition between ADAR1^{p150} and RIPK3 in binding ZBP1 may exist. While the ZBP1-ADAR1^{p150} complex does not induce cell death, the ZBP1-RIPK3 complex induces PANoptosis. For ZBP1 to interact with RIPK3, it should sense Z-NAs via its Zα domain. Since ADAR1^{p150} also senses Z-NAs, there may be competition between ADAR1 and ZBP1 for binding to Z-NAs. Binding of Z-NAs by ADAR1 leads to the formation of modified NAs, which may fail to be efficiently sensed by NA sensors including ZBP1. ADAR, adenosine deaminase acting on RNA; NAs, nucleic acids; RIPK3, receptor-interacting serine/threonine-protein kinase 3; RHIM, receptor-interacting protein homotypic interaction motif; ZBP1, Z-DNA binding protein 1; Z-NAs, Z-nucleic acids.

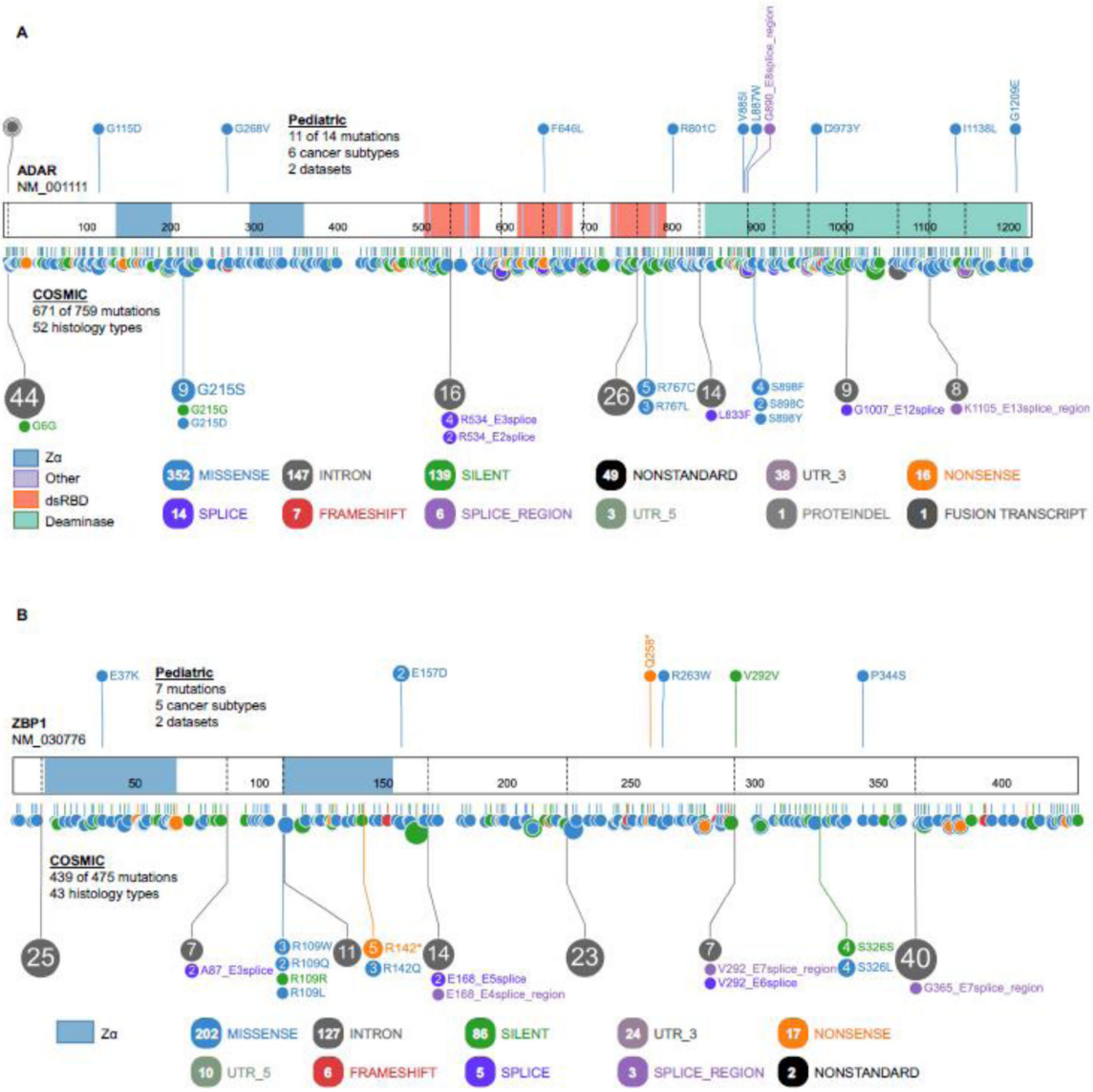

Figure 2: Schematic showing cancer-associated mutations in human ADAR1 and ZBP1

Cancer-associated mutations in the human proteins **A)** ADAR1 (https://pecan.stjude.cloud/proteinpaint/ADAR1) and **B)** ZBP1 (https://pecan.stjude.cloud/proteinpaint/ZBP1) as per the Catalogue of Somatic Mutations in Cancer (COSMIC) database and the Pediatric Cancer Genome Project depicted using ProteinPaint (https://pecan.stjude.cloud/proteinpaint). Each color-coded circle depicts a mutation, and the larger circles with numbers represent mutations found in more than one sample. This interactive tool allows for visualization of major attributes of the mutation, including the details of the sample from which the mutation was identified. ADAR, adenosine deaminase acting on RNA; dsRBD, double-stranded RNA binding domain; UTR, untranslated region; ZBP1, Z-DNA binding protein 1.

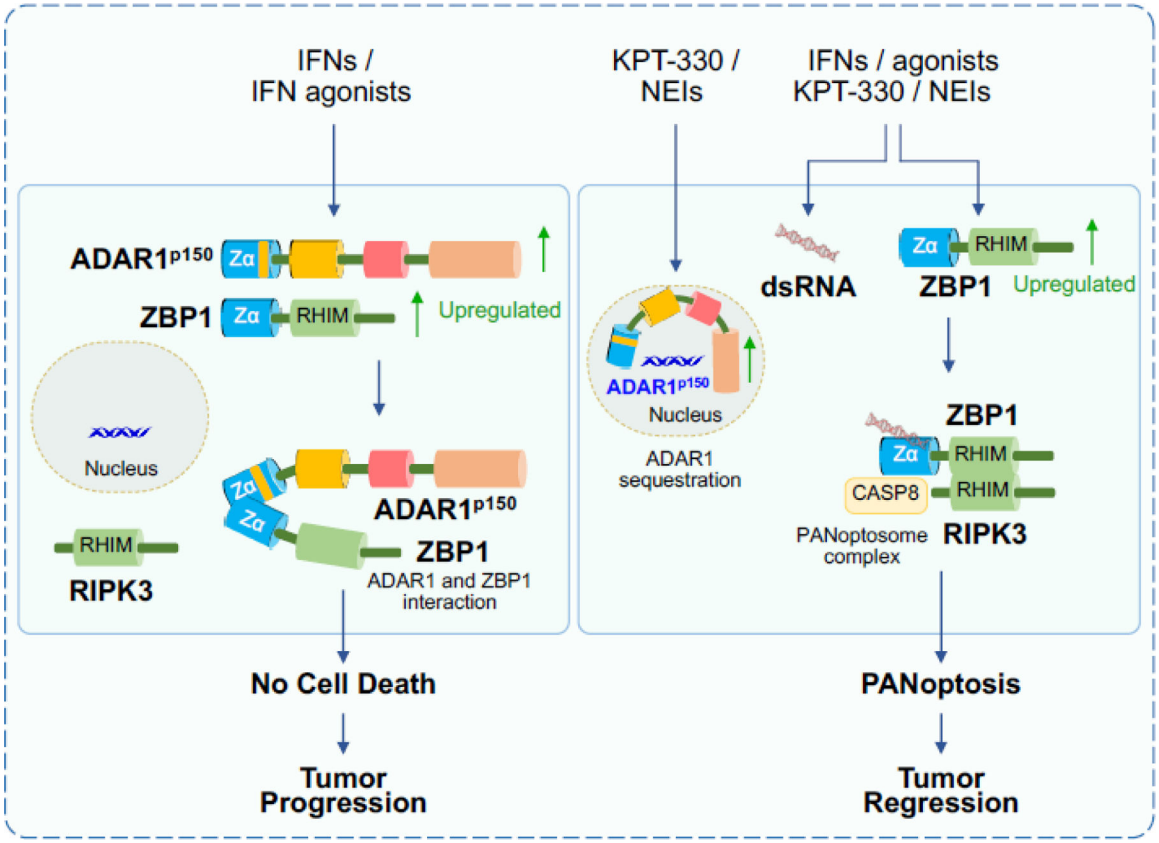

Figure 3: Model of ADAR1 suppression of ZBP1-mediated PANoptosis and its therapeutic modulation for cancer therapy

In mammalian macrophages, IFN or IFN agonists induce expression of both ADAR1^{p150} and ZBP1, which can interact via their Zα domains to induce cell survival and, in the context of cancers, tumor progression. During treatment with NEIs (i.e., KPT-330) and IFNs or their agonists, ADAR1^{p150} is sequestered in the nucleus, causing dsRNA accumulation in the cytosol. The dsRNA is then recognized by the Zα domain of ZBP1, which is followed by the interaction of ZBP1 with RIPK3 via their RHIM domains, leading to the formation of a PANoptosome complex that also contains caspase-8, ASC, NLRP3, and other proteins. The multiprotein PANoptosome executes PANoptosis, which inhibits tumorigenesis ADAR1, adenosine deaminase acting on RNA1; ASC, apoptosis-associated speck-like protein containing a caspase activation and recruitment domain; IFN, interferon; NEI, nuclear export inhibitor; NLRP3, nucleotide-binding oligomerization domain-like receptor (NLR) family pyrin domain-containing 3; RIPK3, receptor-interacting serine/threonine-protein kinase 3; RHIM, receptor-interacting protein homotypic interaction motif; ZBP1, Z-DNA binding protein 1.

Table 1:

Significance of innate immune sensors in the survival and development of ADAR1-deficient mice.

Mouse Model and Survival Outcome	Genetic cross	Survival Outcome of Cross	Reference
Adar1$^{-/-}$ Embryonically lethal [10, 79, 109]	*Mda5*$^{-/-}$	Survive to birth	[80]
	Rigi$^{-/-}$	Embryonically lethal	[80]
	Mavs$^{-/-}$	Survive to birth	[80, 81]
	Sting$^{-/-}$	Embryonically lethal	[80]
	Ifnar1$^{-/-}$	Embryonically lethal	[81]
	Ifnar1$^{-/-}$*Ifngr*$^{-/-}$	Embryonically lethal	[83]
	Stat1$^{-/-}$	Embryonically lethal	[81]
	Zbp1$^{-/-}$	Embryonically lethal	[32]
	Mavs$^{-/-}$*Zbp1*$^{-/-}$	~ 40% survive to adulthood	[32]
	Mavs$^{-/-}$*Zbp1*$^{mZα/mZα}$	~ 50% survive to adulthood	[33]
	Mavs$^{-/-}$*Ripk3*$^{-/-}$	~ 40% survive to adulthood	[32]
Adar1^{p150null} Embryonically lethal [80, 110]	*Mda5*$^{-/-}$	Survive to birth	[80]
	Mavs$^{-/-}$	Survive to birth	[80]
	Zbp1$^{-/-}$	Embryonically lethal	[31]
	Mda5$^{-/-}$*Zbp1*$^{-/-}$	Some survive up to 27 days	[31]
Adar1$^{E861A/E861A}$ Embryonically lethal [16]	*Mda5*$^{-/-}$	Rescued	[16]
	Bak$^{-/-}$*Bax*$^{-/-}$	Embryonically lethal	[85]
Adar1$^{mZα/-}$ Survive to birth [32, 70]	*Mavs*$^{+/-}$	50% survive to at least 15 weeks of age	[32]
	Mavs$^{-/-}$	Rescued	[70]
	Zbp1$^{-/-}$	~70% survive to adulthood	[32]
	Zbp1$^{mZα/mZα}$	~50% survive to adulthood	[32, 33]
	Mavs$^{+/-}$*Zbp1*$^{-/-}$	Rescued	[32]
	Ripk3$^{-/-}$	Early postnatal lethality	[32, 33]
	Mlkl$^{-/-}$	Early postnatal lethality	[32]
	Mlkl$^{-/-}$*Casp8*$^{-/-}$	Early postnatal lethality	[33]
	Ripk3$^{-/-}$*Fadd*$^{-/-}$	Early postnatal lethality	[32]
	Mlkl$^{-/-}$*Fadd*$^{-/-}$	Early postnatal lethality	[32]
	Ripk1$^{mR/mR}$*Mlkl*$^{-/-}$	Early postnatal lethality	[32]
Adar1$^{P195A/p150null}$ Median survival of 25 days [31, 84]	*Zbp1*$^{-/-}$	Rescued	[31]
	Mda5$^{-/-}$	Rescued	[84]
	Lgp2$^{-/-}$	Rescued	[84]
	Pkr$^{-/-}$	Rescued	[84]
	Rnasel$^{-/-}$	No role	[84]
	Mlkl$^{-/-}$	No role	[31]
	Mlkl$^{-/-}$*Casp8*$^{-/-}$	Survive to birth	[31]

Mouse Model and Survival Outcome	Genetic cross	Survival Outcome of Cross	Reference
	Ripk1^{KD}	No role	[31]
	Ripk3$^{-/-}$	Improved survival	[31]

***Adar1*$^{-/-}$** : Global deficiency of both isoforms of adenosine deaminase acting on RNA 1 (ADAR1)

***Adar1*p150null**: Global deficiency of ADAR1^{p150} isoform

***Adar1*E861A/E861A** : Mice with an editing-deficient knock-in mutation (E861A)

***Adar1*mZα/–** : Mice carrying mutations in the Zα domain (N175A and Y179A) in one allele paired with a second *Adar1* null allele

***Adar1*P195A/p150null** : Mice carrying a mutation in the Zα domain (P195A) on one allele paired with deletion of ADAR1^{p150} in the second allele of *Adar1*

***Zbp1*mZα/mZα** : Mice carrying mutations in two Zα domains (N46A and Y50A in Zα1 domain, N122A and Y126A in Zα2 domain) on both alleles of *Zbp1*

***Ripk1*mR/mR** : Mice carrying a mutation in the receptor-interacting protein homotypic interaction motif (RHIM) domain on both alleles of *Ripk1*

***Ripk1*KD** : Mice carrying a receptor-Interacting serine/threonine-protein kinase 1 (RIPK1) kinase dead mutation (K45A) on both alleles of *Ripk1*