

News and Commentary

Distinct roles for the cellular inhibitors of apoptosis proteins 1 and 2

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Cell Death and Disease (2011) 2, e135; doi:10.1038/cddis.2011.20; published online 24 March 2011

The cellular inhibitor of apoptosis proteins 1 and 2 (cIAP1 and cIAP2) are members of a highly conserved and critically important family of inhibitor of apoptosis proteins (IAPs) that function to mitigate both intrinsic and extrinsic death signalling. Their conserved mechanism of action *in vitro* relies on competitive inhibition of caspase activity. In recent years, however, ubiquitin ligase activity within cIAP1/2 has been shown to have a central role in regulating NF- κ B signalling and programmed cell death through the ubiquitylation of key components of TNF receptor complexes. Owing to the importance of NF- κ B signalling in a variety of pathologies, cIAP1/2 have become desirable drug targets and there is thus a strong rationale for studying how cIAP1/2 expression is regulated in these contexts. Although these paralogous proteins function in a redundant manner, their context-dependent differential expression provide for non-redundant roles. Here, we will focus on evaluating possible distinct functions for cIAP1 in specific cellular settings where it acts as the dominant player and review recent advances in our understanding of how cIAP1 expression can be regulated at the post-transcriptional level.

The Emerging Importance of cIAP1/2 in Modulating NF- κ B Signalling and Programmed Cell Death

cIAP1, cIAP2 and the X chromosome-linked IAP (XIAP) are members of the family of inhibitor of apoptosis proteins (IAPs) that were initially reported to function as competitive inhibitors of caspases through their baculoviral IAP repeat (BIR) domains.¹ Although all three proteins directly bind and inhibit caspases, cIAP1/2 were found to only weakly exhibit this activity *in vivo*.² Instead, cIAP1/2 have been found to demonstrate functional redundancy by virtue of E3 ubiquitin ligase activity within their RING domains that targets specific substrates for proteasomal degradation (K48-polyubiquitin linkages), or for participation in specific signalling pathways (K63-polyubiquitin linkages).³

Defining the gamut of cIAP1/2 ubiquitin ligase substrates has uncovered a critical role for IAP ubiquitin ligase activity in modulating inflammatory signalling pathways. The cellular

response to the inflammatory cytokine TNF- α is the best understood of these pathways and serves to signal cell survival through activation of NF- κ B transcription factors, or cell death through apoptosis or programmed necrosis (necroptosis). Upon binding of TNF- α to the TNF receptor 1, the adaptor proteins TRADD, TRAF2 and/or TRAF 5 are recruited along with cIAP1 and/or cIAP2 and the RIP1 kinase (Figure 1a). RIP1 serves as a substrate for cIAP1 or cIAP2-mediated K63-polyubiquitylation that signals recruitment of the TAK/TAB complex and subsequent activation of NF- κ B. The cIAP1/2-mediated polyubiquitylation of RIP1 in the TNF- α -stimulated cell also prevents internalisation of complex I that would lead to the formation of complex II, activation of caspase-8 and death by apoptosis. If caspase 8 activity is limiting, or if the activity of RIP1 or its binding partner RIP3 is high, complex III forms and leads to programmed necrosis (necroptosis) (Figure 1a). Thus, the absence of cIAP1/2 renders cells sensitive to TNF- α -induced death. Paradoxically, removal of either cIAP1 or cIAP2 in unstimulated cells enhances constitutive NF- κ B signalling, as the result of NIK stabilisation and induction of both the classical and alternative arms of the NF- κ B response.⁴

Specialised Roles for cIAP1?

As the activities of cIAP1 and cIAP2 appear to be redundant, one may ask in which cellular contexts could these proteins have distinct roles? Confounding this question is the observation that some cell types exhibit a strong compensatory mechanism, whereby reducing cIAP1 levels leads to an increase in cIAP2 expression. This phenomenon was first observed in spleen and thymus isolated from cIAP1 knockout animals and is attributed to the ability of cIAP1 to target cIAP2 for ubiquitin-mediated degradation.⁵ Surprisingly, cIAP1 is not a target for degradation by cIAP2, as cIAP2 knockout animals show unchanged expression of cIAP1.⁶ This compensatory mechanism does not appear to be a universal event. For example, reduction in the levels of cIAP1 by RNAi enhances cIAP2 expression in breast cancer cells (MDAMB231), but not

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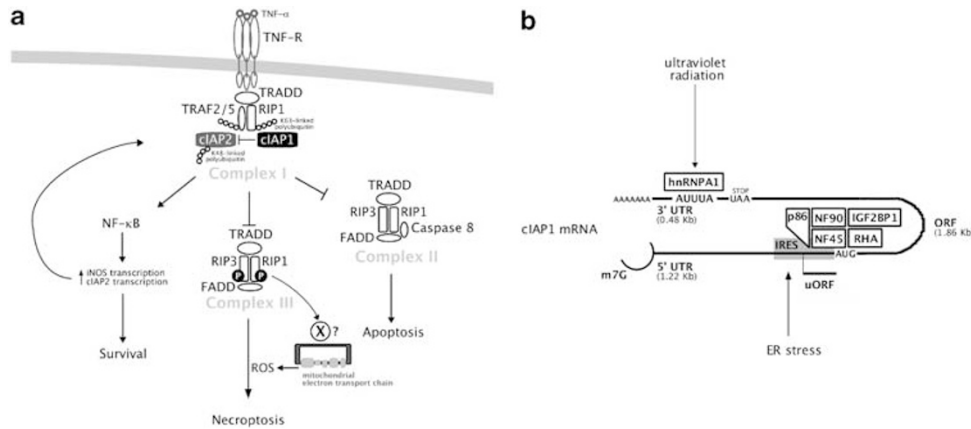


Figure 1 (a) The central role of cIAP1 in NF- κ B signalling and programmed cell death. Binding of tumour necrosis factor alpha (TNF- α) to its plasma membrane receptor (TNFR) invokes assembly of TNF- α receptor associated death domain (TRADD), RIP1 kinase (RIP1), TNFR associated factor 2 or 5 (TRAF2/5), and cIAP1 or cIAP2 that together form TNFR Complex I. The E3 ubiquitin ligase activity of cIAP1 can act to buffer high levels of cIAP2 protein through K48-linked polyubiquitylation and degradation of cIAP2. cIAP1 has a critical role in activating the NF- κ B pathway through K63-linked polyubiquitylation of RIP1 kinase and/or TRAF2 or 5. The propagation of the NF- κ B signal into the nucleus induces expression of a number of pro-survival genes including cIAP2 and inducible nitric oxide synthetase (iNOS). The lack of cIAP1 ubiquitin ligase activity leads to receptor internalisation and the formation of death-inducing cytosolic complexes mediated by deubiquitylated RIP1. Specifically, the RIP1- and RIP3-containing Complex II can form to activate caspase 8, leading to cleavage and inactivation of RIP1 and RIP3 and the initiation of an apoptotic cascade. Alternatively, in the absence of caspase-8 or in a context where deubiquitylated RIP1 or RIP3 activity is high, the 'necrosome' (complex III) can form, in which mutual and auto-phosphorylation of RIP1 and RIP3 (labelled 'P' in this diagram) leads to propagation of necrotic signals and programmed necrotic cell death. Reactive oxygen species (ROS) are an important mediator of necroptosis and cIAP1 limits RIP1-dependent production of ROS through the mitochondrial electron transport chain. As RIP1 and RIP3 do not translocate to mitochondria in the absence of cIAP1, an unknown factor (labelled 'X' in the diagram) must be responsible for increased mitochondrial redox activity. (b) cIAP1 mRNA sequence features and post-transcriptional regulatory nodes. A diagram of the capped (m7G) and polyadenylated cIAP1 mRNA illustrating the size (in kilobases, kb) and location of the 5' and 3' untranslated regions (UTRs). This diagram is not to scale. RNA binding proteins have been found to alter cIAP1 mRNA stability and translation. Specifically, ultraviolet radiation induces nucleocytoplasmic translocation of hnRNP A1 and binding to AU-rich elements in the 3'UTR, leading to decreased cIAP1 mRNA stability. An IRES that enhances translation of cIAP1 mRNA following endoplasmic reticulum (ER) stress is 150 bp long and is located proximal to the initiation codon (AUG) of the main open reading frame (ORF). An upstream ORF (uORF) whose CUG initiation codon bisects the IRES codes for a non-functional peptide of 21 amino acids and acts to repress cIAP1 mRNA translation under normal physiological conditions. Several RNA binding proteins or IRES trans-acting factors (ITAFs) have been implicated in the regulation of cIAP1 translation including p86 nuclear factors 90 and 45 (NF90 and NF45, respectively), insulin-like growth factor 2 binding protein 1 (IGF2BP1), and RNA helicase A (RHA)

in embryonic kidney cells (HEK293T). Further, the general lack of correlation between levels of cIAP1 and cIAP2 across a panel of cancer cell lines⁷ implies that the oft-cited compensatory mechanism might prove to be the exception rather than the rule. Here, we will highlight two recent studies that have uncovered a starring role for cIAP1 in specific cellular contexts.

Death by any other name. TNF- α signalling normally leads to transcriptional activation of cIAP2 in a bid to subvert apoptosis. However, Vanlangenakker *et al.* found that the L929 mouse fibrosarcoma cell line sustains programmed necrosis (rather than apoptosis) following treatment with TNF- α and unexpectedly expresses very low levels of cIAP2.⁸ The cIAP2 levels were also unaffected in TNF- α -activated cells transfected with cIAP1 siRNA. This is in stark contrast to other cell types where compensation by cIAP2 protein in a cIAP1-null background has been demonstrated.⁵ Reducing cIAP1 expression using the small-molecule cIAP1/2 antagonist BV6 or by cIAP1 RNAi renders these cells sensitive to TNF- α -mediated necrosis.⁸ The increased sensitivity was found to be due to enhanced activity of RIP1 kinase in cIAP1-depleted cells and thus an increase in the levels of the 'necrosome' – a necroptosis-specific complex (complex III in Figure 1a) that is analogous to the caspase-8-activating complex II that forms during TNF- α -mediated apoptosis. An additional surprise in this study was that cIAP1 is able to inhibit production of necrosis-inducing mitochondrial reactive oxygen species (ROS) in a RIP-dependent manner suggesting a unique role for

cIAP1 in mitochondrial metabolism. The authors found that RNAi-mediated downregulation of the mitochondrial redox carrier complex I (a component of the mitochondrial electron transport chain) in the absence of cIAP1 prevented RIP1-dependent ROS production. Interestingly, this did not involve translocation of either RIP1 or RIP3 to mitochondria suggesting the involvement of an unknown factor in this pathway (Figure 1a). Thus, in this specific context, cIAP1 expression is necessary and sufficient to keep the brakes on RIP1-dependent necroptosis owing to the lack of compensatory cIAP2 expression.

cIAP1 is in the kNOW. In examining cIAP1-null mice and their response to intra-tracheal bacterial infection, Prakash *et al.*⁹ found the expected cIAP2 compensation present in lung tissue. However, examination of peritoneal macrophages from these same mice revealed no upregulation of cIAP2. The lack of cIAP activity in these macrophages illustrates that mechanisms preventing compensation by upregulation of cIAP2 exist *in vivo*, although we do not yet have any insight into their nature. Perhaps not surprisingly, a defect in proliferative activity and inflammatory response of cIAP1-null peritoneal macrophages rendered the mice resistant to LPS-induced endotoxin shock. This finding is similar to that observed in cIAP2-null mice,⁶ but cIAP1-null macrophages were also unable to produce and release nitric oxide (NO). This striking finding, due to impairment in their ability to express

inducible NO synthetase (iNOS), establishes a new and specialised role for cIAP1 in the innate immune response.

These examples of distinct regulation and specialised roles for cIAP1 in pathological contexts highlight its potential as a target for the development of drug therapies that modulate its expression. In addition, the key role that cIAP1 has in cell survival, tumorigenesis and cancer progression coupled with frequent cancer-associated mutations in IAP alleles gives a strong rationale for studying the regulation of cIAP1 and cIAP2 expression. Although post-transcriptional regulation of cIAP2 has been described,¹⁰ its expression is primarily controlled at the level of transcription in an NF- κ B-dependent manner, and at the level of the proteasome by cIAP1.⁵ In contrast, as outlined below, expression of cIAP1 is tightly controlled through modulation of stability and translation of its mRNA.

cIAP1: The Paradigm of Post-transcriptional Control

Regulation of cIAP1 mRNA stability. cIAP1 mRNA half-life is significantly reduced following exposure to ultraviolet radiation (UVR). The resultant decrease in cIAP1 expression leads to a significant increase in NF- κ B activity.¹¹ We found that the stability of cIAP1 mRNA is mediated by AU-rich elements (ARE) in its 3'UTR. AREs are cis-acting elements containing an AUUUA pentamer (or similar sequences), and generally target the mRNA for degradation through the exosome.¹² Although the exact mechanism through which cIAP1 mRNA is degraded remains unknown, we identified hnRNP A1 as a protein factor that is responsible for cIAP1 mRNA instability (Figure 1b). hnRNP A1 is known as a modulator of splicing¹³ and IRES-dependent translation.¹⁴ Like many RNA binding proteins, hnRNP A1 is primarily a nuclear protein although it accumulates in the cytoplasm following specific environmental cues. For example, osmotic stress causes hnRNP A1 to accumulate in the cytoplasm where it represses translation of specific mRNAs¹⁴ or associates with stress granules.¹⁵ UVR was found to induce a similar cytoplasmic accumulation of hnRNP A1, resulting in increased association of hnRNP A1 with cIAP1 mRNA and its subsequent degradation.¹¹

Regulation of cIAP1 mRNA translation. cIAP1 protein expression is also repressed as the result of both a long (1.2 kb) 5'UTR that hinders ribosomal movement, and a small upstream open reading frame (uORF) that strongly attenuates initiation of cIAP1 translation at the authentic AUG start codon (Figure 1b; Warnakulasuriarachchi *et al.*¹⁶). In response to stress, however, translation of cIAP1 is mediated by the internal ribosome entry site (IRES), which is active following induction of endoplasmic reticulum (ER) stress or arsenite treatment and results in enhanced expression of cIAP1.^{16–18} cIAP1 IRES activity is regulated by specific trans-acting protein factors including p86, a protein formed by cleavage of p97/DAP5 during ER stress.¹⁹ Using RNA affinity chromatography, additional cIAP1 IRES binding proteins NF45, NF90, IGF2BP1 and RHA were identified (Figure 1b; Graber *et al.*²⁰). NF45 was further shown to be required for induction of cIAP1 during the ER stress response. This data suggests the existence of an auxiliary translation initiation complex that is active during

times when the cell has reduced general protein synthesis allowing for continued translation of this important regulator of apoptosis and NF- κ B signalling.

cIAP1 post-transcriptional regulatory nodes therefore represent druggable targets. Although we do not yet understand the cellular mechanism that prevents compensation by cIAP2 in the cell contexts explored herein, targeting cIAP1 expression in such situations represents a tractable strategy for the development of drug therapies that modulate programmed cell death and/or the innate immune response.

Conflict of interest

The authors declare no conflict of interest.

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