IAPs: from caspase inhibitors to modulators of NF-κB, inflammation and cancer

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Abstract | The realization that alterations in inhibitor of apoptosis (IAP) proteins are found in many types of human cancer and are associated with chemoresistance, disease progression and poor prognosis, has sparked a worldwide frenzy in the development of small pharmacological inhibitors of IAPs. The development of such inhibitors has radically changed our knowledge of the signalling processes that are regulated by IAPs. Recent studies indicate that IAPs not only regulate caspases and apoptosis, but also modulate inflammatory signalling and immunity, mitogenic kinase signalling, proliferation and mitosis, as well as cell invasion and metastasis.

The ultimate goal of all current cancer therapies, including radiotherapy, chemotherapy and immunotherapy, is the destruction of the cancer cell. The effects of most therapeutic agents and the success of current therapeutic intervention schemes heavily rely on the ability of a cancer cell to engage its own cell death programme. This exposes a conundrum as one of the hallmarks of a cancer cell is the acquired ability to dampen and/or circumvent the engagement of pro-death programmes[1]. Therefore, the same mutations that permit tumour formation by suppressing cell death will also reduce treatment sensitivity and inevitably contribute to treatment failure and relapse.

This has provided the necessary incentive for the development of more specific approaches to re-establish an apoptotic programme in cancer cells. A promising approach is the development of small therapeutic compounds, referred to as Smac mimetics, that are designed to block the function of members of the inhibitor of apoptosis (IAP) protein family, and these are currently in clinical trials for the treatment of cancer[2] (TABLE 1). Alterations in IAPs are found in many types of human cancer and are associated with chemoresistance, disease progression and poor prognosis[2,3]. Consistent with the idea that different types of cancer cells are addicted to IAPs for their survival, the inactivation of IAPs, particularly when combined with other treatments, results in the death of most tumour cells, at least under tissue culture conditions[4–9]. Furthermore, inactivation of IAPs does not seem to be detrimental to normal cells. Paradoxically, however, loss of IAPs is also associated with the development of certain types of cancer. Therefore, depending on cellular context, IAPs seem to have both pro-tumorigenic and anti-tumorigenic roles[10].

IAPs were thought to function primarily by regulating caspases, which are cysteine proteases that are involved in apoptosis. However, IAPs also influence a multitude of other cellular processes, such as ubiquitin (Ub)-dependent signalling events that regulate activation of nuclear factor-κB (NF-κB) transcription factors, which in turn drive the expression of genes important for inflammation, immunity, cell migration and cell survival. Further, IAPs reportedly modulate signalling events that promote the activation of cell motility kinases and metastasis[11,12]. Moreover, they regulate mitogenic kinase signalling, proliferation and mitosis (BOX 1). Many of these cellular processes are frequently deregulated in cancer and contribute directly or indirectly to disease initiation, tumour maintenance and/or progression[2,3]. In this Review, we discuss new insights into the cancer-related roles of IAPs. In particular we focus on celluar IAP1 (cIAP1; encoded by *BIRC2*), cIAP2 (encoded by *BIRC3*) and XIAP (encoded by *BIRC4*). The biological functions of the other BIR-containing proteins NAIP (encoded by *BIRC1*), Survivin (encoded by *BIRC5*), BRUCE (encoded by *BIRC6*), MLIAP (encoded by *BIRC7*) and ILP2 (encoded by *BIRC8*) are covered elsewhere[13] and are not the subject of this Review.

The IAP tool box

The defining feature of an IAP protein is the presence of the baculovirus IAP repeat (BIR) domain, a

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At a glance

- Alterations in inhibitor of apoptosis (IAP) proteins are prevalent in many types of human cancer and are associated with chemoresistance, disease progression and poor prognosis.
- IAPs are best known for their ability to regulate caspases; however, IAPs also influence a multitude of other cellular processes.
- Possibly the most important contribution of IAPs to cell survival and tumorigenesis resides in the ability of cIAP1, cIAP2 and XIAP to regulate ubiquitin-dependent activation of nuclear factor-κB (NF-κB) and innate immune responses.
- Constitutive activation of NF-κB and chronic inflammation both have a major role in tumour development and are seen in most tumour types, including leukaemia, lymphomas and solid tumours.
- NF-κB can be activated through the canonical and non-canonical signal transduction cascade, and cIAPs are crucial regulators of both these pathways.
- cIAPs are also indispensable in protecting cancer cells from the lethal effects of tumour necrosis factor receptor 1 activation.
- Small-molecule IAP antagonists, termed Smac mimetics, cause the rapid depletion of cIAPs and show potent anti-tumorigenic activity *in vitro* and *in vivo*.

Intrinsic apoptosis pathway
This pathway is dependent on mitochondria and is activated by developmental cues and cellular stresses such as DNA damage and oncogene activation. Pro-apoptotic BCL-2 family proteins facilitate the release of cytochrome *c* and other apoptogenic factors from the mitochondrial intermembrane space.

Extrinsic apoptosis pathway
This pathway is initiated on ligation of cell surface receptors of the TNFR superfamily. Activation of these receptors triggers the assembly of death-inducing signalling complexes that serve as a platform to activate caspase 8 and caspase 10.

zinc-binding fold of approximately ~70 amino acid residues that mediates protein–protein interactions[14–16] (BOX 1), and is essential for the anti-apoptotic potential of most IAPs. IAPs, of which there are eight in humans, carry between one and three copies of this domain (FIG. 1). The mammalian IAPs, XIAP, cIAP1 and cIAP2, contain three such domains in their amino-terminal portion. These IAPs also harbour additional domains such as the carboxy-terminal RING finger domain that provides them with Ub ligase (E3) activity[17]. Moreover, they carry a Ub-associated (UBA) domain through which they interact with ubiquitylated proteins[18,19]. Finally, cIAP1 and cIAP2 contain a caspase-recruitment domain (CARD), the function of which is currently unknown.

Although BIR domains generally mediate interactions with other proteins, it is important to note that individual BIR domains possess specific binding characteristics. On the basis of the presence of a deep peptide-binding groove, BIR domains can be approximately grouped into type I and type II domains (BOX 1). Type I BIR domains lack a peptide-binding groove, or possess a shallow pocket only, whereas type II BIRs carry a distinctive hydrophobic cleft through which they bind to N-terminal tetrapeptides called IAP-binding motifs (IBMs)[20–22]. Apoptosis-regulatory IAPs such as XIAP, cIAP1, cIAP2 and *Drosophila melanogaster* IAP1 (DIAP1) and DIAP2 carry two such type II BIR domains. Apart from DIAP1, all these IAPs also carry a type I BIR domain[20]. These domains do not bind caspases or IAP antagonists but use distinct modes to interact with an altogether different set of proteins. For example, the type I BIR (BIR1) of cIAPs mediates binding to tumour necrosis factor receptor (TNFR)-associated factor 1 (TRAF1) and TRAF2, whereas XIAP BIR1 mediates interaction with transforming growth factor-β (TGFβ)-activated kinase (TAK1) binding protein, TAB1 (REFS 23–25).

Table 1 | **IAP antagonists in clinical trials**

Organization	Compound name	Condition	Clinical stage
Genentech	GDC-0152	Locally advanced or metastatic solid malignancies, or non-Hodgkin's lymphoma without leukaemic phase	Phase I
Novartis Pharmaceuticals	LCL161	Advanced solid tumours	Phase I
TetraLogic Pharmaceuticals	TL32711	Solid tumours and lymphomas	Phase I
Ascenta Therapeutics	AT-406	Advanced solid tumours and lymphomas	Phase I
Human Genome Sciences	HGS1029	Advanced solid tumours and lymphomas	Phase I

IAP, inhibitor of apoptosis.

Apoptotic pathways

The key destructive molecules of apoptosis are the caspases, a family of specific cysteine proteases[26]. Generally, caspases are ubiquitously expressed as inactive zymogens, however, in response to specific death stimuli, caspases are activated in cascades of auto-stimulation and trans-stimulation[27] (BOX 2).

IAPs as direct caspase inhibitors. Of all IAPs, mammalian XIAP is the only IAP that functions as a direct caspase inhibitor in a strict biochemical sense[28]. Other IAPs, such as DIAP1, DIAP2, cIAP1 and cIAP2, are inefficient in directly inhibiting caspases *in vitro*. Overexpression of XIAP efficiently inhibits caspase activation and apoptosis stimulated by the intrinsic apoptosis pathways and extrinsic apoptosis pathways[29–34]. Conversely, cells that lack XIAP are sensitized to apoptosis[8,35–37]. Cancer cells can propagate under adverse conditions such as nutrient limitation, hypoxia, oncogene deregulation, DNA damage and chromosomal aberrations — circumstances that trigger caspase activation and apoptosis in normal cells. In some cases, this failure of cancer cells to engage caspases might be because of deregulated expression and activity of IAPs and this could increase the apoptotic threshold. In this respect, IAPs seem to have crucial roles in tumour maintenance and resistance to chemotherapy treatments, although IAP expression on its own is clearly not the only determinant that increases the apoptotic threshold and enables tumour growth[2,3,38,39].

XIAP can directly bind and inhibit caspase 3, caspase 7 and caspase 9. Residues in the linker region between the BIR1 and BIR2 domain of XIAP bind to the active site pocket of caspase 3 and caspase 7 (REFS 16,40–44) (FIG. 2). In particular, several of these caspase-binding residues are conserved in other IAPs, including cIAP1, cIAP2 and DIAP2, which is most similar to XIAP. Insertion of the BIR1–BIR2 linker region of XIAP into the catalytic pocket of active effector caspases occludes substrate entry and results in the inhibition of the catalytic activity of the caspase. Surprisingly, the BIR2 domain has little direct role in the inhibitory mechanism, as the linker region preceding the BIR2 domain makes almost all inhibitory contacts. Nevertheless, the BIR2 domain is functionally important as it makes additional contact with an IBM motif present at the neo-amino-terminus of the caspase subunit[44,45].

Neo-amino-terminus
On proteolytic cleavage of a polypeptide, the amino acid immediately C terminal to the cleavage site becomes the new N terminal amino acid of the C terminal fragment.

In non-active caspases, this motif is hidden, but becomes exposed following cleavage-mediated activation of caspase 3 and caspase 7. The concerted binding of XIAP to the catalytic pockets and IBMs of these effector caspases substantially strengthens caspase binding, and is essential for XIAP to effectively dock onto effector caspases for inhibition. The strategy through which XIAP inhibits caspase 9 is fundamentally different (FIG. 2). Here, the BIR3 domain of XIAP binds to the homodimerization surface of caspase 9 (REFS 22,46). Caspase 9 requires a dimerization-induced conformational change to generate a productive catalytic pocket, and XIAP interferes with caspase 9 dimerization.

XIAP-mediated inactivation of caspase 3, caspase 7 and caspase 9 does not require a functional RING finger under *in vitro* conditions or when overexpressed[26,47]. However, recent evidence indicates that this might be different under physiological conditions. *In vivo*, endogenous XIAP reportedly requires a functional RING to exert its full anti-apoptotic potential[48]. Gene targeting techniques have been used to create an endogenous *Birc4* gene that lacks the RING finger (XIAPΔ^{RING}). This sensitizes fibroblasts to TNFα-induced cell death and leads to increased rates of apoptosis in an Eμ-MYC mouse model of B cell lymphoma. In both these systems, XIAPΔ^{RING}-mutant cells respond in the same way as XIAP-null cells. Moreover, following death stimuli, caspase activity is significantly higher in XIAPΔ^{RING} cells than in wild-type controls, suggesting that the BIR domains alone are not sufficient to block caspase activity *in vivo*. However, these observations are also consistent with a role for the RING finger of XIAP in regulating survival signalling pathways, such as signalling to NF-κB (see below)[49], that impinge on the sensitivity of cells to apoptotic triggers. Therefore, additional experiments are needed to fully clarify the mechanisms by which the RING finger domain of XIAP contributes to cell survival *in vivo*.

Box 1 | **BIR domains and IAP equality**

Inhibitor of apoptosis (IAP) proteins are classified by the presence of one or more baculovirus IAP repeat (BIR) domains. BIR domains can be grouped into type I and type II BIR domains on the basis of the presence or absence of a deep peptide-binding groove. Type I BIR domains do not possess this groove and associate with a different range of proteins than the type II BIRs[23–25]. Type II BIR domains predominantly associate with proteins that carry an amino terminal IAP-binding motif (IBM). The most prominent feature of IBMs is the presence of an N terminal alanine or serine, which must be exposed and unblocked. This inserts into the extensive hydrophobic cleft on the surface of type II BIRs and forms hydrogen bonds with neighbouring residues[21,22,121] (FIG. 1). An arginine side chain in the third position provides favourable interactions with the hydrophobic moiety in the BIR binding pocket, and hydrophobic residues in the second and fourth positions also contribute to IBM-mediated BIR binding. This firmly anchors IBM-carrying proteins to IAPs. Subtle changes in the peptide-binding groove of type II BIR domains alter the preference for particular IBMs[157]. Therefore, different type II BIR domains have distinct binding preferences for specific IBM-carrying proteins. Thus, the BIR2 of XIAP binds to the IBMs of caspase 3 and caspase 7, and the BIR3 binds to the IBM of caspase 9 (REFS 22,44–46). XIAP, cIAP1, cIAP2, *Drosophila melanogaster* IAP1 (DIAP1) and DIAP2 carry two such type II BIR domains. The tandem arrangement of these type II BIRs increases the repertoire of proteins with which these IAPs can interact and enhances the binding affinity to IBM-carrying proteins that form dimers or trimers, such as Smac[158].

Ubiquitin-mediated regulation of caspases. The strongest genetic evidence for a role of the RING finger domain, and Ub conjugation, in regulating caspases and apoptosis comes from studies in *D. melanogaster*[50–65], in which DIAP1 has a pivotal role in cell viability[51]. DIAP1 regulates apoptosis by directly binding to the caspase 9 homologue DRONC (also known as caspase Nc) and the caspase 3-like effector caspases drICE and DCP1 (REFS 50,53,60) (FIG. 2). Although the IAP–caspase association is a decisive step in the regulation of apoptosis in *D. melanogaster*, physical interaction between DIAP1 and caspases alone is insufficient to regulate caspases *in vivo*. In addition to binding, DIAP1 requires RING finger-mediated Ub ligase activity to neutralize caspases. Mutations in the RING finger of DIAP1 that abrogate its Ub ligase activity, but not caspase binding, cause a severe loss-of-function phenotype[52,56]. Following binding, the RING is required to ubiquitylate and inactivate the initiator caspase DRONC, and the effector caspases drICE and DCP1 (REFS 56,63). The precise mechanism through which polyubiquitylation inactivates DRONC is controversial and might involve degradative as well as non-degradative inactivation mechanisms[66].

The functional consequence of caspase ubiquitylation is clearer for drICE and DCP1. Although the precise mechanism of inhibition requires structural verification, the conjugated Ub chains seem to sterically occlude the catalytic pocket of the caspases, thereby interfering with substrate entry[63]. IAP-mediated ubiquitylation of caspases is not restricted to *D. melanogaster*, as XIAP, cIAP1 and cIAP2 also reportedly ubiquitylate caspase 3 and caspase 7, targeting them for either monoubiquitylation[67] or polyubiquitylation[42,68]. Although the functional consequence of caspase monoubiquitylation remains unexplored, polyubiquitylation of caspase 3 and caspase 7 has been linked to their degradation[42,68] and non-degradative inactivation[48].

Caspase-independent survival signalling

Probably the most important contribution of IAPs to cell survival and tumorigenesis is the ability of several IAPs to regulate NF-κB signal transduction and innate immune responses. A large body of evidence indicates that constitutive activation of NF-κB and chronic inflammation have a major role in tumour development and are seen in most tumour types, including leukaemia, lymphomas and solid tumours[69–71]. NF-κB transcription factors are important regulators of the genes necessary for innate and adaptive immune responses and for the survival and proliferation of certain cell types. Moreover, NF-κB also controls cell growth and motility. The realization that IAPs function as crucial components of various NF-κB signal transduction pathways has sparked renewed activities in studying the role of IAPs in health and disease.

Ubiquitin-dependent regulation of NF-κB. Transcription factors of the NF-κB and Rel family are activated in response to receptor stimulation and various intracellular stressors, including DNA damage[72,73]. NF-κB is a family of transcription factors that consists of RELA (also known as p65), RELB, CREL and the precursor and ankyrin repeat-containing proteins NF-κB1

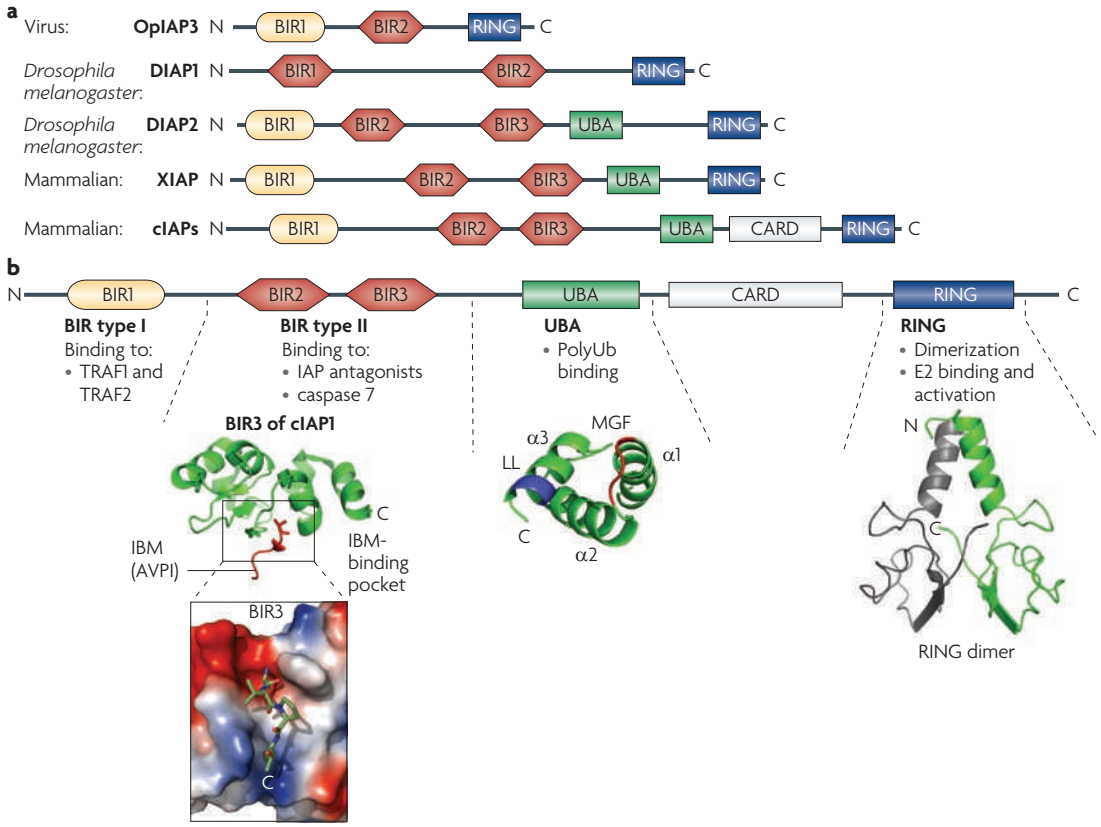

Figure 1 | **Family association and domain characteristics. a** | The first inhibitor of apoptosis (IAP) protein, OpIAP, was identified from a baculovirus strain in 1993 by Miller and colleagues[168], on the basis of its ability to suppress virus-induced apoptosis of infected cells. Cellular IAPs were subsequently identified in insects and vertebrates. Several of the IAPs discussed in this Review are depicted schematically. In mammalian IAPs, baculovirus IAP repeat (BIR) domains enable interactions with proteins. The BIR domains of IAPs can be grouped into type I (yellow) and type II (red) BIR domains on the basis of the presence or absence of a deep peptide-binding groove (BOX 1). **b** | Proteins such as caspases and IAP antagonists interact with type II BIR domains, whereas tumour necrosis factor receptor-associated factor 1 (TRAF1) and TRAF2 interact with type I BIR domains. The structure of the cIAP1-BIR3 peptide-binding groove bound to the amino-terminal portion of the IAP antagonist Smac is shown (AVPI; red). The AVPI structure was modelled into the groove of BIR3. The ubiquitin (Ub)-binding UBA domain binds polyubiquitin (polyUb). The structure of the IAP2 UBA domain is a prediction. The amino acid residues MGF and LL (shown in red and blue, respectively) of the UBA domain form the hydrophobic interaction surface that mediates binding to polyUb chains. The function of the caspase-recruitment domain (CARD), which generally serves as a protein interaction surface, is unknown. The carboxy-terminal RING domain is required for Ub ligase activity, and is a dimerization interface and docking site for ubiquitin conjugating enzymes (E2s). The structure of a cIAP2 RING dimer is shown; the grey and green stuctures depict two respective cIAP2 molecules[169].

(p105, which is processed to generate p50) and NF-κB2 (p100, which is processed to generate p52). NF-κB1 and NF-κB2 do not contain transactivation domains, and they rely on their interaction with RELA, RELB or CREL to positively regulate transcription. Depending on the mechanism leading to NF-κB activation, NF-κB signal transduction can be roughly classified into the canonical pathway and the non-canonical pathway[72,73]. Importantly, both these pathways are Ub-dependent signal transduction cascades in which Ub ligases, Ub receptors and deubiquitylating enzymes (DUBs) build up, recognize and remove Ub signals, allowing the temporally controlled assembly of protein complexes that lead to the activation of kinases that regulate NF-κB[74,75].

The initial genetic evidence for a role of IAPs in regulating NF-κB came from *D. melanogaster*, in which DIAP2 was found to be essential for TAK1–TAB2- or TAB3-mediated activation of NF-κB transcription factors in the immune deficiency (*Imd*) signalling cascade[76–78] — a cascade that bears a striking resemblance to the signalling cascade that is induced by activation of TNFR1. In the absence of DIAP2, flies fail to activate NF-κB, which is needed to drive the expression of antimicrobial peptide genes and to mount an innate immune reaction in response to infection by Gram-negative bacteria. DIAP2-mediated signalling to NF-κB depends on its Ub ligase activity. In conjunction with the E2 Ub-conjugating enzymes UBC5 and UBC13–UEV1A, DIAP2 is thought to promote the conjugation of K63-linked Ub chains on IMD[79]. These chains then serve as scaffolds for Ub receptors to allow Ub-dependent recruitment and activation of downstream kinases, ultimately leading to the phosphorylation and activation of NF-κB. IAP-mediated activation of NF-κB is also crucial

Canonical pathway
Signals that predominantly activate the RELA–p50 heterodimers.

Non-canonical pathway
Signals that result in the activation of RELB–p52 heterodimers.

Box 2 | **Caspase-mediated cell death (apoptosis)**

Caspases are synthesized as inactive zymogens that become activated through internal proteolysis or by induced proximity. Apoptotic caspases are classified into initiator caspases with long pro-domains and effector caspases with short pro-domains. In mammals, the initiator caspases, caspase 8 (and its close homologue, caspase 10) and caspase 9, are activated by signal-induced dimerization in multiprotein complexes known as a death-inducing signalling complex (DISC) and apoptosome, respectively[159]. DISCs are formed on the ligation of death receptors (DRs), which constitute a subset of cell surface receptors of the tumour necrosis factor receptor (TNFR) superfamily. Activation of TNFR1 by TNF leads to oligomerization of the receptor and formation of a multiprotein complex consisting of TNFRSF1A-associated via death domain (TRADD), TNFR-associated factor 2 (TRAF2), TRAF5, cellular inhibitor of apoptosis 1 (cIAP1), cIAP2, RIPK1 and additional proteins. By mechanisms that are not clearly understood, this complex is released from the receptor and the death domain of TRADD is able to interact with Fas-associated death domain (FADD), which in turn recruits caspase 8 and caspase 10 through their death effector domain[94,160]. Through induced proximity, caspase 8 and caspase 10 are activated and may process downstream effector caspase 3 and caspase 7 as well as the pro-apoptotic BCL-2 family protein BID. The caspase 8 processed BID (tBID) induces a conformational change of the multi-domain BCL-2 family proteins BAX and BAK, which leads to their insertion into the outer mitochondrial membrane, the permeabilization of the outer membrane and the release of apoptogenic factors, including cytochrome *c*, Smac (also known as DIABLO), OMI (also known as HTR2A) and apoptosis-inducing factor (AIF), into the cytosol. In the cytosol, cytochrome *c* binds APAF1 and facilitates the formation of the apoptosome — a heptameric complex of APAF1 and caspase 9. Within this complex, caspase 9 becomes activated owing to induced proximity, and in turn activates caspase 3 and caspase 7 through proteolytic cleavage between the large and small subunits. These activated effector caspases then orchestrate the execution phase of apoptosis through limited proteolysis of multiple substrates. The mitochondrial apoptosis pathway can also be activated by numerous non-receptor stimuli, including genotoxic stress and oncogene activation[160].

in zebrafish. Using a forward-genetic approach, the gene encoding zebrafish cIAP1, *birc2*, was identified as an essential positive regulator of TNFR-mediated activation of NF-κB[80]. Loss of *birc2* results in deficient NF-κB signalling, and activation of a caspase 8-dependent apoptotic programme that leads to vascular haemorrhage and blood pooling. This phenotype, which is referred to as the *tomato* phenotype, can be rescued by microinjection of *Ikkγ* mRNA. As overexpression of IKKγ can result in NF-κB activation, this result suggests that the *tomato* phenotype is caused by defective NF-κB signalling. Zebrafish cIAP1-mediated signalling to NF-κB relies on functional RING finger and UBA domains[18].

cIAP1, cIAP2 and XIAP are also implicated in modulating NF-κB activation and inflammatory signalling. Notably, NF-κB reportedly induces the expression of cIAP1, cIAP2 and XIAP, thereby promoting NF-κB activation in a positive feedback loop. XIAP is implicated in the activation of NF-κB in response to DNA damage and bacterial infection[49,81,82]. Although little is known about how XIAP mediates signalling to NF-κB under physiological conditions, mechanistic studies based on the ectopic expression of XIAP suggest that XIAP recruits TAK1 through BIR1-mediated binding of the TAK1 adaptor protein TAB1. This facilitates the dimerization and activation of TAK1 (REF. 25) (see below). In addition, functional RING and UBA domains are required, but how these contribute to NF-κB activation remains unclear[18,83]. XIAP also mediates NF-κB activation by promoting degradation of COMMD1, a negative regulator of NF-κB[84,85]. Reportedly, XIAP also functions as a cofactor in TGFβ and bone morphogenetic protein (BMP) signalling, and mechanistic studies indicate that the ectopic expression of XIAP stimulates SMAD4-dependent transcription from JUN N-terminal kinase (JNK)- and TGFβ-responsive promoters[86,87]. However, whether XIAP is rate-limiting for these signalling pathways remains to be determined.

A flurry of recent studies has indicated that cIAP1 and cIAP2 have a particularly important role in regulating canonical and non-canonical NF-κB signalling[4,5,80,88–93]. cIAPs regulate these pathways in opposite directions: cIAP1 and cIAP2 are essential positive regulators of the canonical pathway and are required to suppress constitutive activation of non-canonical NF-κB signalling. Therefore, overexpression as well as loss of cIAPs can result in deregulated NF-κB activation, tumour cell survival and chemoresistance[4–6,10,18].

Canonical NF-κB pathway. The role of cIAPs in activating the canonical pathway in response to TNFR1 has been studied in detail. Binding of trimeric TNFα to TNFR1 triggers the recruitment of the adaptor protein TNFRSF1A-associated via death domain (TRADD), the Ub ligases TRAF2, TRAF5, cIAP1 and cIAP2, and the protein kinase RIPK1 (FIG. 3). This membrane-localized complex is frequently referred to as complex-I[94]. cIAP-mediated ubiquitylation of the components of complex-I, such as RIPK1, stimulates Ub-dependent recruitment of the dimeric linear ubiquitin chain assembly complex (LUBAC), which is composed of HOIL1 and HOIP, and the kinase complexes TAK1–TAB2–TAB3 and IKKγ–IKKα–IKKβ[74]. Ub-binding domains that are present in HOIP[95], TAB2 (REF. 96) and IKKγ[97] mediate the recruitment of LUBAC, TAK1–TAB2–TAB3 and IKKγ–IKKα–IKKβ, respectively. Once tethered to the Ub chains of complex-I, LUBAC promotes the synthesis of polyUb chains on IKKγ, as well as other components of complex-I[95,98], resulting in the stabilization of complex-I. Ubiquitylation also supports additional recruitment, retention, ubiquitylation and activation of IKKγ–IKKα–IKKβ by TAK1 (REF. 95). IKKβ phosphorylates NF-κB inhibitor-β (NF-κBIβ), targeting it for Ub-dependent proteasomal degradation, allowing NF-κB to translocate to the nucleus where it drives the expression of target genes.

Until recently it was thought that TRAF2 and UBC13 functioned as the Ub ligase and E2-conjugating enzyme, respectively, that target RIPK1 for K63-linked polyubiquitylation. However, recent data indicate that TRAF2 and TRAF1 function as adaptors to recruit cIAPs to the TNFR1 signalling complex, and that cIAPs function as the apical Ub ligases for TNFR1-mediated activation of NF-κB[89,92,95,99–101]. Notably, cIAP1 and cIAP2, which are highly similar proteins, seem to function redundantly in the relay of the TNFα signal. Accordingly, *Birc2*- and *Birc3*-single knockout mice are asymptomatic and do not display noticeable defects in TNFR1-mediated ubiquitylation of RIPK1 and activation of NF-κB[102,103]. However, loss of both of these cIAPs strongly impairs TNFα-mediated NF-κB activation[89,90,92,95].

cIAP-mediated ubiquitylation of the components of complex-I is also essential to protect cells from the lethal effects of TNFα[6,104,105]. Unlike CD95 ligand, TNFα does not kill cells in most circumstances, and instead stimulates NF-κB and MAP kinases, leading to cell survival and stimulation of inflammation. Notably, although ubiquitylation of RIPK1 is thought to be a crucial step in Ub-mediated activation of NF-κB, a recent report suggests that RIPK1 is not essential for TNFα-induced activation of NF-κB, at least in mouse cells[106]. Consistently, *Ripk1*$^{-/-}$ MEFs are not particularly sensitive to TNFα. In the absence of cIAPs, however, TNFα stimulates the formation of a secondary cytoplasmic complex, complex-II, which contains RIPK1, Fas-associated via death domain (FADD) and caspase 8 (REFS 6,104). This complex is formed within 2 hours of stimulation, is entirely RIPK1-dependent and derives from the plasma membrane-bound complex-I following its detachment from TNFR1 (FIG. 3). In the absence of cIAPs, formation of complex-II results in the rapid activation of caspase 8 and induction of apoptosis. In most cases, this form of death is completely blocked by caspase inhibitors, indicating that it is executed by caspases[4–6]. However, in some cells, exposure to caspase inhibitors or genetic defects that prevent caspase 8 activation switches the apoptotic response to necrosis[107–111]. The switch to necrotic cell death depends on the levels of RIP3, as RIP3 is recruited to complex-II and phosphorylates and activates RIPK1, thereby promoting necrosis[109,110,112]. Interestingly, cIAPs have a similar function in regulating the cells response to CD95 ligation. cIAPs prevent the recruitment of RIPK1 and the formation of a RIPK1-dependent complex-II that triggers programmed necrosis following CD95 stimulation[113].

Together, these data indicate that cIAPs function at a pivotal step in death receptor signalling. The involvement of cIAPs in maintaining cell survival is consistent with their role in the Ub-dependent formation of complex-I, recruitment of LUBAC, stabilization of complex-I and efficient activation of NF-κB. Additionally, cIAPs also contribute to cell survival by preventing the formation of a RIPK1-dependent, caspase 8-activating complex by ubiquitylating RIPK1 at the receptor complex.

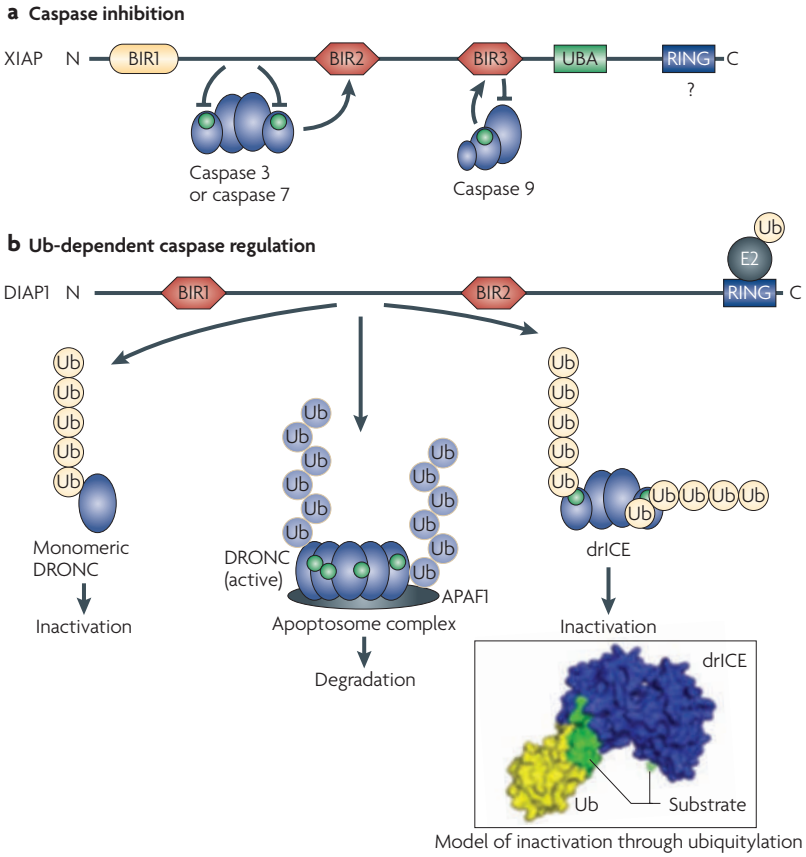

Figure 2 | **IAP-mediated regulation of caspases: inhibition versus Ub-dependent inactivation. a** | XIAP directly inhibits the effector caspase 3 and caspase 7, and the initiator caspase 9. The sequence preceding the BIR2 domain of XIAP occupies the catalytic pocket of caspase 3 or caspase 7, thereby blocking substrate entry. In addition, the BIR2 domain interacts with the inhibitor of apoptosis (IAP)-binding motif (IBM) of caspase 3 or caspase 7 that is exposed following their proteolytic activation (shown in green). XIAP-mediated inhibition of caspase 9 requires proteolytic cleavage of caspase 9, which exposes an IBM that binds to the BIR3 of XIAP. Caspase 9 activity is blocked because XIAP prevents caspase 9 dimerization, a prerequisite for initiator caspase activity. The RING domain of XIAP does not contribute to caspase binding and, therefore, is not required for caspase inhibition *in vitro*. **b** | IAP-mediated regulation of caspases as exemplified by the *Drosophila melanogaster* IAP1 (DIAP1), which is an essential negative regulator of the initiator caspase DRONC and the effector caspases drICE and DCP1. Direct physical interaction with drICE or DCP1 and DRONC is mediated through the BIR1 and BIR2 domains of DIAP1, respectively. The BIR2–DRONC association is essential for DIAP1 to neutralize DRONC but mere binding alone is not sufficient. Following binding, the RING finger of DIAP1 promotes ubiquitin (Ub) conjugation of caspases. Several Ub-dependent inactivation mechanisms have been suggested and include non-degradative ubiquitylation of monomeric DRONC that suppresses activation; limitation of spontaneous apoptosome formation by targeting apoptosome-associated DRONC for degradation; and suppression of effector caspases through non-degradative Ub conjugation. Potentially, deubiquitylating enzymes may remove Ub chains following exposure to cell death stimuli causing rapid caspase reactivation. A predicted structure of drICE is shown ubiquitylated at K178 (for simplicity only one Ub moiety (yellow) is shown). Ub conjugation interferes with substrate binding (the substrate peptide is indicated in green), suggesting steric hindrance as potential mechanism of Ub-mediated caspase inhibition.

Non-canonical NF-κB pathway. Non-canonical activation of NF-κB predominantly occurs in response to ligands of the TNF receptor superfamily such as CD40L, B cell activating factor (BAFF) and TWEAK[114]. This pathway depends on the inducible phosphorylation and proteasome-mediated partial degradation of the NF-κB family member NF-κB2 to its p52 form (FIG. 4). This process is regulated by the NF-κB-inducing kinase (NIK) and IKKα, but not IKKβ or IKKγ[114]. NIK phosphorylates IKKα at Ser176 and Ser180, and NF-κB2 at Ser866 and Ser870. This recruits IKKα heterodimers, which phosphorylate additional residues in the N and C termini of NF-κB2, leading to the ubiquitylation and proteasome-mediated processing of NF-κB2 to its mature form, p52 (REF. 115). Non-canonical activation of NF-κB is normally shut down in resting cells owing to the constitutive degradation of NIK through an Ub–ligase complex consisting of TRAF3–TRAF2–cIAP1 and/or cIAP2 (REFS 91,116). TRAF3 directly binds to NIK and recruits it to TRAF2–cIAP1 through its ability to heterodimerize with TRAF2 through its C terminal TRAF domain. TRAF2 and TRAF3 both have a RING finger domain; however, the Ub ligase activity of cIAP1

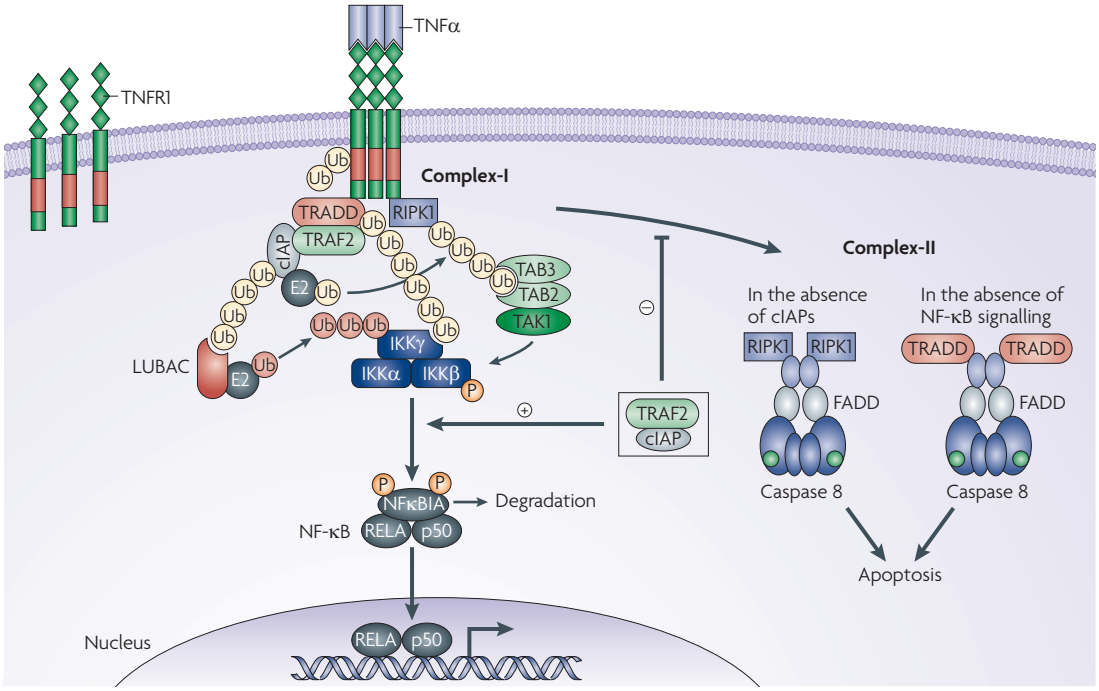

Figure 3 | **IAPs function as E3 ligases in TNFR1-mediated activation of NF-κB.** Activation of tumour necrosis factor receptor 1 (TNFR1) stimulates the formation of complex-I that consists of TNFR1, TNFR-associated via death domain (TRADD), RIPK1, TNFR-associated factor 2 (TRAF2) and cellular inhibitor of apoptosis 1 (cIAP1). cIAPs ubiquitylate (through K63-linked chains, shown in cream) several components of this complex, which causes ubiquitin (Ub)-dependent recruitment of linear ubiquitin chain assembly complex (LUBAC), transforming growth factor-β (TGFβ)-activated kinase (TAK1)–TAB2–TAB3 and IKKγ–IKKα–IKKβ through their respective Ub-binding domains (UBDs). Once recruited, LUBAC conjugates M1-linked Ub chains (shown in pink) on to IKK, and probably other components of complex-I. In addition to stimulating TNFα-mediated activation of nuclear factor-κB (NF-κB), cIAPs also suppress the formation of the death-inducing complex-II, which is the activation platform for caspase 8 that induces death by the extrinsic pathway. In the absence of cIAPs, this complex is formed by RIPK1, but in the absence of NF-κB activation it is formed by TRADD. FADD, Fas-associated via death domain; NF-κBIA, NF-κB inhibitor-α.

and cIAP2 is responsible for the conjugation of degradative K48-linked polyUb chains to NIK. TRAF2 and TRAF3 seem to function as adaptor proteins only: recruiting NIK to cIAPs. Genetic or pharmacological deletion of TRAF2, TRAF3 or cIAPs prevents NIK turnover and results in the accumulation of NIK protein levels, causing spontaneous activation of NIK and stimulation of non-canonical NF-κB signalling.

Activation of non-canonical NF-κB signalling is induced in response to the activation of a subset of TNFR superfamily members that includes CD40, BAFF receptor (BAFFR) and TNFRSF12A[114,117]. Although it is clear that cIAPs are required for receptor-mediated activation of the non-canonical pathway, the precise mechanism through which this is achieved is not fully understood, and might vary depending on the receptor[91,93,116]. Although different receptors use distinct mechanisms to deplete the components of the TRAF3–TRAF2–cIAP Ub ligase complex, the overall outcome is the same: the liberation and stabilization of NIK, which results in the spontaneous activation of the non-canonical pathway.

The idea that NIK levels are kept low by the TRAF3–TRAF2–cIAP Ub ligase complex explains why genetic deletion of TRAF2, TRAF3 or cIAPs is sufficient to cause NF-κB2 activation. It also explains how certain cancer types achieve constitutive activation of NF-κB. For example, 20% of patients with multiple myeloma have genetic lesions that drive unrestrained non-canonical NF-κB signalling[10,118]. Strikingly, biallelic deletions of *BIRC2* and *BIRC3*, *TRAF2*, *TRAF3* and *CYLD* were among the most frequent genetic alterations identified. Moreover, enhanced expression of CD40, lymphotoxin-β receptor (LTβR), *TNFRSF13B*, *NFKB2* and *NIK* is also frequently found[10,118]. Mutations causing increased levels of NIK and non-canonical activation of NF-κB might not be exclusive to multiple myeloma — breast[119] and pancreatic cancers[120] might also have mutations that result in the activation of the non-canonical pathway. The identification of multiple genetic alterations affecting genes that are involved in regulating NIK levels provides evidence that unrestrained non-canonical NF-κB signalling substantially contributes to tumorigenesis. As IAPs are essential for preventing the accumulation of NIK and unrestrained NF-κB signalling, it seems likely that IAPs suppress NIK-mediated tumour development in some cell types.

Inhibition of IAPs as anti-cancer therapy

To date more than 50 patents have been filed that are aimed at blocking IAPs and pushing cancer cells into apoptosis. The observation that a short IBM tetrapeptide can bind and block the interaction between type II

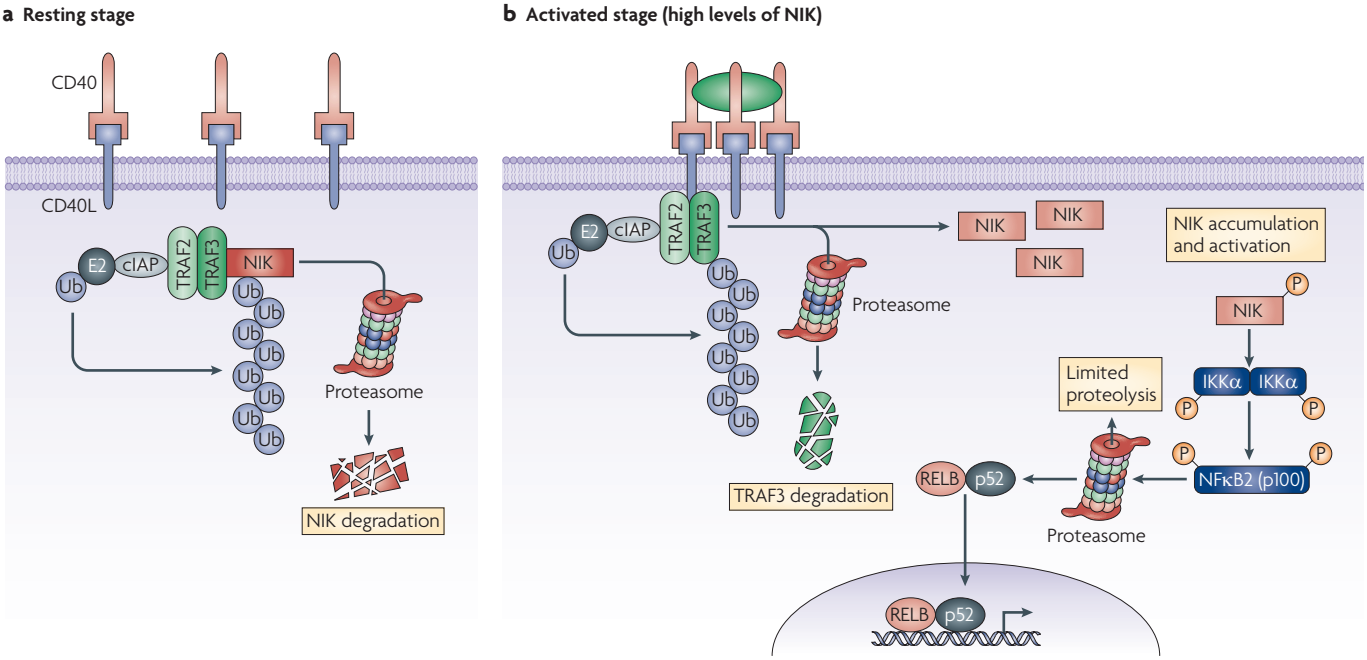

Figure 4 | **Regulation of non-canonical NF-κB signalling. a** | Under resting conditions cellular inhibitor of apoptosis (cIAP) proteins target nuclear factor-κB (NF-κB)-inducing kinase (NIK) for ubiquitylation and proteasomal degradation. cIAP-mediated degradation of NIK requires tumour necrosis factor receptor associated factor 2 (TRAF2) and TRAF3. TRAF3 functions as an adaptor that directly binds to NIK, and recruits it to the TRAF2–cIAP complex through its ability to heterodimerize with TRAF2. **b** | Engagement of CD40 with its ligand CD40L results in the recruitment of the TRAF3–TRAF2–cIAP complex to the receptor. At the receptor, TRAF3 undergoes cIAP-dependent K48-linked polyubiquitylation (Ub) that targets it for proteasomal degradation. In the absence of TRAF3, NIK protein levels accumulate as it can no longer be recruited to the TRAF2–cIAP complex. As NIK levels increase, NIK presumably becomes activated by autophosphorylation (P). Subsequently, NIK activates IKKα, which in turn phosphorylates NF-κB2. This stimulates limited proteasome-mediated proteolysis of NF-κB2 to p52. Removal of the carboxy-terminal ankyrin repeats from NF-κB2 releases the p52–RELB heterodimer, allowing its translocation to the nucleus where it instigates the expression of NF-κB target genes.

BIR domains and caspases[21,121–124], led to the development of small pharmacological drugs mimicking the N terminal IBM motif (AVPI) of mature Smac (also known as DIABLO), a member of the loosely defined family of IAP antagonists (BOX 3; FIG. 1). These compounds selectively bind to the type II BIR domains of numerous IAPs. Most Smac mimetic compounds are dimers resembling the higher-order architectural structure of IAP antagonists[2]. Although originally designed to inactivate XIAP, Smac mimetics are most effective with cIAP1 and cIAP2. Within minutes of exposure, Smac mimetics trigger auto-ubiquitylation and proteasomal degradation of cIAP1 and cIAP2 — although cIAP2 is depleted with slower kinetics than cIAP1in most cases[4,5,104]. This results in the spontaneous activation of non-canonical NF-κB, NF-κB-mediated enhancement of TNFα production and autocrine stimulation of TNFR1. It is clear that the depletion of cIAPs causes a dramatic reduction in RIPK1 ubiquitylation[89,90,92] and the formation of a RIPK1-dependent activation platform that binds and activates caspase 8, causing cell death. Apoptotic or necrotic cell death that is induced by Smac mimetics and TNFα crucially depends on RIPK1, as *Ripk1*-deficient MEFs and cancer cells in which RIPK1 is depleted are resistant to TNFR1 killing following treatment with a Smac mimetic[4–6,88,89,106].

Although several cancer cell lines are sensitive to Smac mimetics alone, most cancer cell lines seem to be resistant[6,125]. Such 'Smac mimetic-resistant cells' survive because they fail to produce TNFα. However, when supplied with exogenous TNFα, these cells also rapidly succumb to TNFR1-mediated apoptosis. Currently, it is not clear why certain cells respond to Smac mimetics by producing TNFα and others do not. Notably, both resistant and sensitive cells have comparable levels of non-canonical NF-κB signalling following Smac mimetic-mediated depletion of cIAPs. *In vivo* this distinction may be of little relevance as malignant cancer cells are flooded with TNFα that is produced by the tumour microenvironment. Therefore, it is expected that *in vivo* many types of tumour cells are sensitive to Smac mimetic treatment. Thus, the difference between Smac mimetic-resistant and Smac mimetic-sensitive tumour cells may predominantly apply to *in vitro* culture settings. Importantly, Smac mimetics are well tolerated *in vivo* and do not seem to sensitize normal primary cells to TNFα-induced killing, which provides a promising therapeutic window[4,6,126–128]. Currently, five Phase I clinical trials are under way assessing the safety, tolerability and pharmacokinetic profiles of Smac mimetics in patients with advanced solid tumours and lymphomas (TABLE 1).

cIAPs and cancer-related inflammation

Although the loss of IAPs favours the development of multiple myeloma, the genomic amplification of 11q22, which contains *BIRC2* and *BIRC3*, occurs at high frequency in hepatocellular carcinoma (HCC), lung and pancreatic cancers, oral squamous cell carcinomas, medulloblastomas and glioblastomas[129–134]. Moreover, recurrent amplification of *Birc2* and *Birc3* also frequently occurs in mouse models of *MYC*-driven liver cancer and spontaneous osteosarcomas[135,136]. In both these tumour models, high levels of cIAP1 expression were required to sustain the rapid growth of amplicon-containing tumours[135,136]. Although it has been postulated that cIAP1 cooperates with MYC by targeting MAD1, a cellular antagonist of MYC, for K48-linked polyubiquitylation and proteasomal degradation[137], it seems likely that cIAP1 also modulates the cellular response to TNFα that is produced by the tumour microenvironment (FIG. 5). In this respect it is interesting to note that the activation of MYC induces the expression of numerous chemokines, which are attractants for inflammatory cells[138] that are thought to support tumour development by releasing growth, trophic and chemotactic factors[139]. In particular, mast cells produce a large amount of vascular endothelial growth factor A (VEGFA), fibroblast growth factor 2, matrix metalloproteinase 9 (MMP9) and TNFα[139]. Emerging evidence indicates that TNFα is one of the key mediators of cancer-related inflammation that drives tumour development and/or progression[140,141]. Consistently, constitutive production of TNFα from the tumour microenvironment is seen in many malignant tumours, and is frequently associated with a poor prognosis[142]. Given that cIAP1 and cIAP2 function as key mediators of TNFα-induced activation of NF-κB and protect cells from the lethal effects of TNFα, it seems likely that increased levels of IAPs at least partly support cancer cell survival by modulating their response to TNFα. Clearly further work is required to fully elucidate how cIAPs contribute to the development and/or progression of various types of cancers.

IAP-mediated regulation of metastasis

The dissemination of tumour cells to distant organs and their proliferation at these sites is one of the hallmarks of tumour progression, and is invariably associated with a fatal disease outcome. Metastatic cells need to suppress the cell death programme following detachment from the trophic environment and extracellular matrix as well as acquire the ability to migrate, pass through basement membranes, invade blood vessels and colonise distant sites[1].

Two recent reports link several mammalian IAPs to tumour cell invasion and metastasis. The physical association of XIAP with survivin was found to drive NF-κB activation, which in turn leads to increased autocrine production of fibronectin, signalling by β1 integrins and activation of the cell motility kinases focal adhesion kinase (FAK) and SRC[11]. This results in tumour cell invasion *in vitro* and metastatic dissemination *in vivo*. Importantly, the role of XIAP in regulating metastasis seems to be independent of its ability to modulate cell survival through caspase inhibition. IAP-mediated regulation of cell invasion and metastasis is not restricted to XIAP–survivin but also extends to cIAPs. Accordingly, targeted knock down of cIAP1, similar to knock down of XIAP or survivin, inhibits MDA-MB231 and PC3 cell invasion. This suggests that controlling tumour cell invasion is a general property of multiple IAPs.

Although high IAP expression levels can contribute to cell migration and metastasis, Rajalingam and colleagues find that XIAP, cIAP1 and cIAP2 actually suppress cell motility *in vitro* in response to growth factor stimulation[12]. XIAP, cIAP1 and cIAP2 bind to CRAF and target it for proteasomal degradation. Accordingly, small interfering RNA-mediated downregulation of XIAP, cIAP1 or cIAP2 stabilizes CRAF and enhances cell migration in a CRAF-dependent manner. Clearly, future studies will need to clarify the precise cellular state and microenvironmental contexts in which XIAP, survivin, cIAP1 and cIAP2 function as genes that either support or suppress metastasis.

Regulation of innate immune responses

Inflammatory responses are mostly beneficial but can also become detrimental if deregulated. It is now recognized that chronic situations, in which unresolved inflammation causes tissue damage and triggers incessant tissue repair and remodelling, strongly contributes to a multitude of human diseases, including cancer[69,143]. Importantly, tissue stress and malfunction, a key characteristic of human tumours, also induces an inflammatory response that is similar to that elicited by invading microbes. Particularly, the necrotic death of tumour cells potently stimulates tissue-resident macrophages. Consequently, tumour-associated necrosis stimulates macrophage recruitment and triggers tissue repair and remodelling, which in turn fuels tumour growth.

MALT lymphoma. The development of MALT lymphoma is a prime example of how the inability to resolve an inflammatory response can contribute to cancer. MALT lymphoma, which is the most common extra-nodal non-Hodgkin B cell lymphoma, arises in the mucosa, primarily in the gastric tract and lungs, and is closely associated with chronic infection by *Helicobacter pylori*[144]. Initially, inflammation-associated

Box 3 | **IAP antagonists**

Antagonists of the inhibitor of apoptosis (IAP) proteins are characterized by the presence of an alanine at position 1 that anchors these proteins to the surface of specific baculovirus IAP repeat (BIR) domains of IAPs (FIG. 1). IAP antagonists have little in common apart from this feature. The activities of the *Drosophila melongaster* IAP antagonists, Reaper, Grim and Hid, are essential for apoptosis during development[161]. Cells that lack these proteins fail to activate the apoptosis programme, just like cancer cells. In normal *D. melanogaster* cells these proteins are either not expressed or post-transcriptionally silenced by microRNAs or MAPK activity[162–164]. In mammalian cells, IAP antagonists are constitutively expressed but sequestered to mitochondria (Smac (also known as DIABLO) and OMI (also known as HTRA2)) or the endoplasmic reticulum (GSPT1; also known as eRF3), from where they are released to the cytosol on receipt of a death stimulus[123,124,165,166]. IAP antagonist proteins predominantly function by binding to the BIR domains of IAPs and blocking their access to caspases, and possibly many other molecules. In addition, some IAP antagonists, such as Reaper, Grim and Hid, also stimulate IAP auto-ubiquitylation and proteasomal degradation[54,57,167].

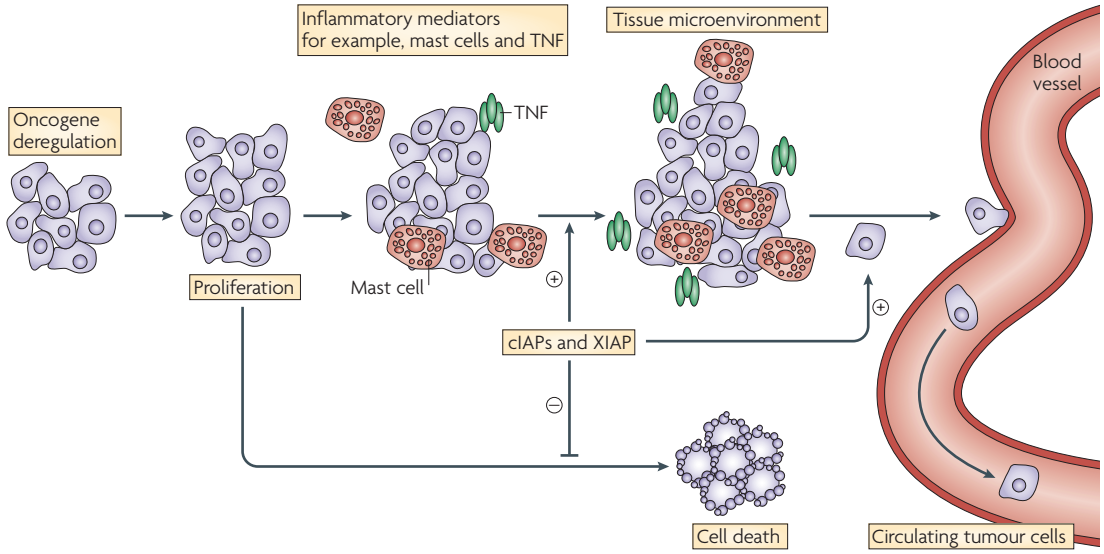

Figure 5 | **IAPs in oncogenesis.** Cellular inhibitor of apoptosis 1 (cIAP1) synergises with MYC in tumorigenesis and this might be partly through its ability to protect cancer cells from the lethal effects of tumour necrosis factor-α (TNFα) produced by the tumour microenvironment. Deregulation of MYC in tumour cells stimulates the expression of numerous chemokines that are attractants for inflammatory cells, such as mast cells, that are essential for macroscopic tumour expansion. Inflammatory cells are thought to support tumour development by releasing growth, trophic and chemotactic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor 2 and TNFα. As cIAPs suppress TNFα-induced cell death, it is likely that increased levels of cIAPs support cancer cell survival by modulating their response to TNFα. cIAPs and XIAP are thought to contribute to cancer cell invasion and metastasis through their ability to drive nuclear factor-κB (NF-κB)-mediated expression of genes involved in cell motility, migration and invasion.

tumours are infection-dependent and regress when patients receive antibiotics. However, such tumours frequently progress to become self-sustaining as a result of genetic rearrangements and no longer respond to antibiotics.

The most prevalent chromosomal aberration associated with MALT lymphoma is the reciprocal translocation of *BIRC3* and *MALT1* (t(11q21:18q21))[144] (FIG. 6). The resulting chimeric oncoprotein consists of the N terminal portion of cIAP2, which includes the three BIR domains and the Ub-binding domain[18], and the C terminal component of the paracaspase MALT1. cIAP2–MALT1 drives B cell transformation and lymphoma progression through constitutive activation of the canonical NF-κB signalling pathway[145]. As the *BIRC3* promoter is a direct downstream target of NF-κB, cIAP2–MALT1-mediated activation of NF-κB results in a positive feedback loop that drives high level expression of cIAP2–MALT1.

Under normal circumstances, MALT1 is an essential intermediate in antigen receptor-mediated activation of NF-κB. Engagement of antigen receptors results in MALT1 oligomerization through its recruitment to a multimeric protein complex that is formed by the adaptor proteins CARMA1 and BCL10 (REF. 75). MALT1 multimerization then recruits and activates the Ub ligase TRAF6, which in turn stimulates K63-linked polyubiquitylation of IKKγ, BCL10, MALT1 and TRAF6 (REFS 146–149). Particularly important is the conjugation of K63-linked polyUb chains on K399 of IKKγ, which results in IKKα and IKKβ activation, and, in turn, activation of NF-κB[147].

In MALT lymphoma, cIAP2–MALT1 bypasses the requirement for BCL10 and upstream signalling events because the first BIR domain of cIAP2 mediates heterotypic oligomerization through binding to the MALT1 C terminus[145,150] (FIG. 6), allowing TRAF6 recruitment, IKKγ ubiquitylation and NF-κB activation. Analysis of t(11q21:18q21)-positive MALT lymphomas indicates that in 98% of all reported cases the breakpoint in *BIRC3* occurs in the intron downstream of exon 7 (REF. 144), which encodes the UBA domain that enables cIAP2–MALT1 to bind to K63- and M1-linked polyUb chains. The specific selection of the breakpoint frequency downstream of exon 7 of *BIRC3* and the consistency of in-frame cIAP2–MALT1 fusions points to a selective advantage for the inclusion of the UBA domain. Consistently, an intact UBA domain is required for trapping ubiquitylated forms of IKKγ, and efficient cIAP2–MALT1-mediated activation of NFkB[18].

Toll-like receptors. Unbalanced production of type I interferons and pro-inflammatory cytokines following the activation of Toll-like receptors (TLRs) contributes to the pathogenesis of autoimmune disease and modulates tumour responses to inflammation[70,143]. Most TLRs are transmembrane receptors that sense derivatives of extracellular microbes through their ligand-binding domains[151]. On stimulation, TLRs trigger the recruitment of adaptor proteins through homotypical protein interactions between Toll and interleukin 1 receptor (TIR) domains that are present in the receptors and adaptors, such as MyD88 and TRIF (FIG. 6). Oligomerization of the receptor and adaptor results in the association

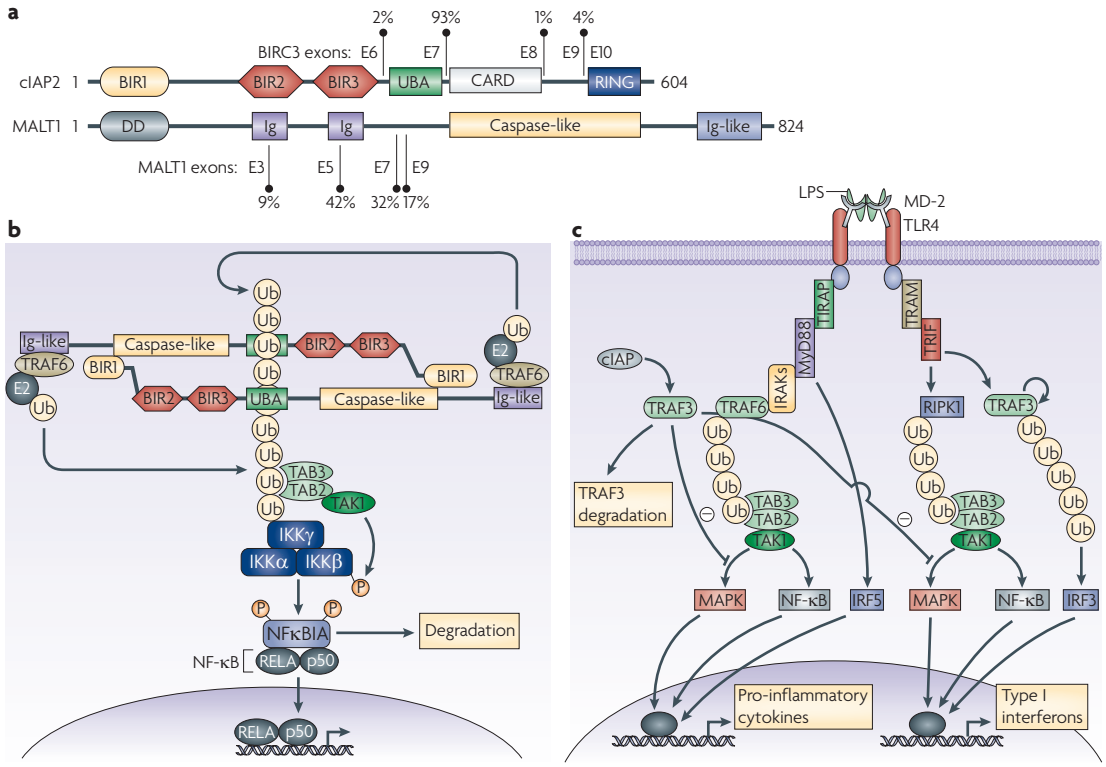

Figure 6 | **IAP-mediated regulation of innate immune responses. a** | The cellular inhibitor of apoptosis 2 (cIAP2)–MALT1 fusion protein drives constitutive activation of nuclear factor-κB (NF-κB) through a mechanism that depends equally on domains contributed by cIAP2 and MALT1. A schematic representation of the position and frequency of the chromosomal breakpoints in *BIRC3* and *MALT1* observed in t(11q21:18q21)-positive MALT lymphomas is shown. Arrowheads show the exon (E) boundaries and position of breakpoints. **b** | Model of cIAP2–MALT1-mediated NF-κB activation: the baculorvirus IAP repeat (BIR) 1 domain of cIAP2 mediates oligomerization of cIAP2–MALT1, permitting the recruitment of tumour necrosis factor receptor-associated factor 6 (TRAF6) and unmodified IKKγ. Following cIAP2–MALT1-assisted polyubiquitylation (Ub) of IKKγ, K63-linked polyubiquitylated IKKγ is tightly bound by cIAP2–MALT1 through the Ub-associated (UBA) domain of cIAP2. This allows the recruitment of transforming growth factor-β-activated kinase 1 (TAK1), activation of the NF-κB kinase complex (IKKα and IKKβ) and subsequent phosphorylation (P) of NF-κBIA leading to its degradation and NF-κB activation. **c** | Binding of lipopolysaccharide (LPS) to the Toll-like receptor 4 (TLR4)–MD2 receptor complex stimulates the MyD88- and TRIF-dependent pathways, which mediate expression of pro-inflammatory cytokines and type I interferons (IFRs). In a stimulus-dependent manner, cIAPs target tumour necrosis factor receptor-associated factor 3 (TRAF3) for proteasomal-mediated degradation. TRAF3 degradation is required to allow MyD88-dependent activation of TAK1 and the production of pro-inflammatory cytokines such as tumour necrosis factor-α (TNFα), interleukin-6 (IL-6) and IL-12β. Notably, LPS-mediated activation of the NF-κB kinase complex and NF-κB seems to be unaffected by the loss of cIAPs. CARD, caspase-recruitment domain; DD, death domain; Ig, immunoglobulin; TAB2, TAK1 binding protein 2.

of additional signalling proteins that trigger the expression of antimicrobial peptides, pro-inflammatory chemokines and cytokines, as well as enzymes that catalyse the production of secondary inflammatory mediators. Of particular importance is the balanced production of interferons and pro-inflammatory cytokines. TLR4-mediated production of interferon and TLR4-mediated production of pro-inflammatory cytokines take different signalling routes and rely on distinct TIR domain-containing adaptors — TRIF and MyD88, respectively[151]. MyD88-dependent production of pro-inflammatory cytokines requires cIAPs, as their depletion resulted in the specific inhibition of production of TNFα, interleukin-6 (IL-6), IL-12α, IL-12β, CXCL2 and CXCL1 (REF. 152). Smac mimetic-mediated inhibition of cIAPs blocks TLR4-mediated and MyD88-dependent activation of MAPK signalling. cIAPs are also required for ligand-induced degradation of TRAF3, which functions as an inhibitor of MAPK activation and inflammatory cytokine production. Therefore, elimination of cIAPs results in the specific inhibition of pro-inflammatory genes without any effect on the anti-inflammatory and tumour-suppressive interferon response[152]. This may be of particular relevance for inflammatory diseases and cancer in which cells propagate in response to pro-inflammatory cytokines, such as TNF, but the growth of which is suppressed by type I interferons[153].

Concluding remarks

Although IAPs were originally identified by their ability to suppress apoptosis, it is now clear that they contribute to cell survival and tumorigenesis by more than simply blocking caspases. Most prominently, many IAPs, such as XIAP, cIAP1 and cIAP2, regulate signalling pathways

that activate NF-κB, which in turn drives the expression of genes involved in inflammation, immunity, cell migration and cell survival. Given that cIAPs protect cancer cells from the lethal effects of TNFα, it is likely that cIAPs also contribute to neoplastic lesions by modulating the response of the cancer cell to TNFα that is produced by the tumour microenvironment. Notably, production of TNFα, which is seen in many malignant tumours, is one of the key mediators of cancer-related inflammation that drives tumour development and/or progression[140,141]. Consistent with the idea that IAPs protect cells from the cytotoxic consequences that are associated with cancer-related inflammation, alterations in IAPs are found in many types of human cancer, and are associated with chemoresistance, disease progression and poor prognosis[2,3]. In such situations, intervention strategies that involve the use of small pharmacological inhibitors of IAPs could have a major therapeutic impact as they deplete cIAPs and leave cancer cells fully exposed to the lethal effects of TNFα. Targeting XIAP in addition to IAPs seems to be beneficial as XIAP suppresses caspases and contributes to metastasis through regulating NF-κB signalling[11,81,82].

However, the discovery that biallelic deletion of *BIRC2* and *BIRC3* leads to constitutive non-canonical NF-κB activation and multiple myeloma highlights the fact that the presence of IAPs can be beneficial, and that loss of IAPs may contribute to carcinogenesis. The presence of frequent biallelic deletions of the cIAP locus in human malignancies is somewhat surprising because such cells are thought to be highly sensitive to TNFα. In multiple myeloma, as in most other malignancies, inflammatory cytokines and growth factors that are produced by the bone marrow microenvironment, as well as recruited leukocytes, contribute to disease progression[154,155]. How cIAP-deficient cells survive and propagate in such an environment is currently unclear. However, this is an important point to clarify as it implies that the prolonged application of Smac mimetics may, under certain settings, support carcinogenesis. Therefore, combination therapies involving Smac mimetics are likely to be safest when used as transient treatment regimens. Such regimens will also have to take into account that inhibition of XIAP may not only render cancer cells more susceptible to apoptosis, and limit inflammatory signalling, but also perturb natural killer T cell homeostasis and contribute to X-linked lymphoproliferative syndrome[156]. Clearly, a deeper understanding of IAP biology, and the situations in which the inhibition of IAP function is beneficial, is needed to limit the potential effects of erroneous sensitization to apoptosis, inadvertent generation of chronic inflammation and/or defects in innate immune signalling.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
UniProtKB: http://www.uniprot.org
caspase 3 | caspase 7 | caspase 9 | cIAP1 | cIAP2 | DIAP1 | DIAP2 | TAB1 | TRAF1 | TRAF2 | XIAP

FURTHER INFORMATION
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