SMAC MIMETICS

a new class of targeted agents that activate apoptotic cell death and block pro-survival signalling in cancer cells

Smac mimetics are a new class of targeted drugs being developed for the treatment of solid tumours and hematologic cancers. Smac mimetics specifically induce apoptotic cancer cell death and block pro-survival signalling in cancer cells. This article provides an overview of the biology of Smac, an endogenous pro-apoptotic molecule, and the development of Smac mimetics as a novel therapeutic approach to treat cancer.

By Dr Mark A. McKinlay

Recent advances in the field of cancer biology have helped to address the challenges of existing cancer therapies and pave the way for new classes of therapeutic agents that target cellular pathways involved in the formation, progression and death of tumour cells. Numerous cancer therapies depend on the tumour cells' ability to undergo apoptosis (programmed cell death). However, tumour cells typically develop a series of mutations that can lead to uncontrolled proliferation and also enable them to evade apoptotic death. Dysregulation of the apoptotic pathways is a major contributor to cancer development and progression, and plays a significant role in cancer resistance to chemotherapies, targeted therapies and radiation. Even if tumours initially respond to these therapies, they often acquire resistance during the course of treatment. Tumour cells can resist apoptosis by increasing expression of proteins that block pro-apoptotic pathways. Overcoming the fundamental mechanisms of cancer resistance and survival, and activating cancer cell death through apoptosis is a focus of current trends in cancer research and drug development. One novel therapeutic approach is the development of small molecule drugs that mimic Smac (second mitochondria-derived activator of caspase), a pro-apoptotic mitochondrial protein that is an endogenous inhibitor of a family of cellular proteins called the Inhibitor of Apoptosis Proteins (IAPs). IAPs regulate apoptosis and cancer cell survival and represent the last line of defence for cancer against cell death by apoptosis. Clinically, IAPs are a key factor in cancer survival, progression and poor prognosis and are associated with tumour resistance to therapies, and as such, are recognised as important therapeutic targets to selectively induce apoptosis in tumour cells. The goal of Smac mimetics (also called IAP antagonists) is to suppress the IAPs, re-establishing the apoptotic pathways and inducing cancer cell death. The unique action of Smac mimetics can also enhance the therapeutic activity of many existing cancer therapies.

This article will provide an overview of the biology of IAPs, its natural inhibitor Smac and the current status of the clinical development of Smac mimetics, which target IAPs in cancer.

Inhibitor of Apoptosis Protein (IAP) Family

The Inhibitor of Apoptosis Proteins (IAPs) are important regulators of cell death and survival.

References

1 LaCasse, EC, Mahoney, DJ, Cheung, HH, Plenchette, S, Baird, S, Korneluk, RG. IAP-targeted therapies for cancer. Oncogene. 2008 Oct 20;27(48):6252-75.

2 Feoktistova, M, Geserick, P, Kellert, B, Dimitrova, DP, Langlais, C, Hupe, M, Cain, K, Macfarlane, M, Häcker, G, Leverkus, M. cIAPs Block Ripoptosome Formation, a RIP1/Caspase-8 Containing Intracellular Cell Death Complex Differentially Regulated by cFLIP Isoforms. Mol Cell. 2011 Aug 5;43(3):449-63. Epub 2011 Jul 7.

3 Tenev, T, Bianchi, K, Darding, M, Broemer, M, Langlais, C, Wallberg, F, Zachariou, A, Lopez, J, MacFarlane, M, Cain, K, Meier, P. The Ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs. Mol Cell, 2011 Aug 5 (3);43:432-458.

4 Ghavami, S, Hashemi, M, Ande, SR, Yeganeh, B, Xiao, W, Eshraghi, M, Bus, CJ, Kadkhoda, K, Wiechec, E, Halayko, AJ, Los, M. Apoptosis and cancer: mutations within caspase genes. J Med Genet. 2009 Aug;46(8):497-510. Epub 2009 Jun 7.

5 Parsons, MJ, Green, DR. Mitochondria in cell death. Essays Biochem. 2010.

6 Mannhold, R, Fulda, S, Carosati, E. IAP antagonists: promising candidates for cancer therapy. Drug Discov Today. 2010 Mar;15(5-6):210-9. Epub 2010 Jan 21.

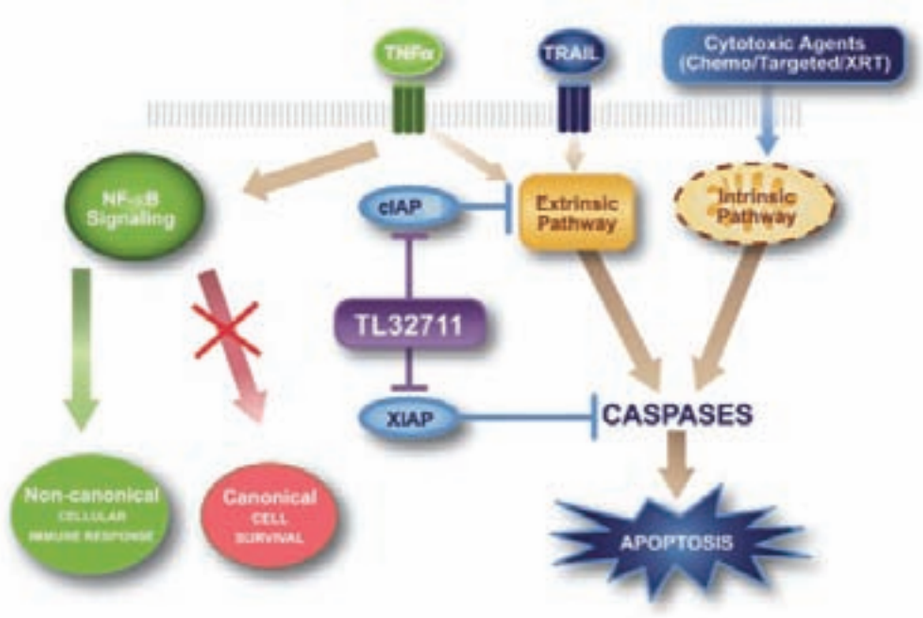
7 Nathan, C, Ding, A. Nonresolving inflammation. Cell. 2010 Mar 19;140(6): 871-82.

8 Karin, M, Greten, FR. NF-κB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol. 2005 Oct;5(10):749-59.

9 Gyrd-Hansen, M, Meier, P. IAPs: from caspase inhibitors to modulators of NF-κB, inflammation and cancer. Nat Rev Cancer. 2010 Aug;10(8):561-74.

Continued on page 39

Smac mimetics: exert anti-tumour activity through four different mechanistic activities



Currently, seven human IAPs have been identified: XIAP, cIAP1, cIAP2, ILP2, BRUCE/Apollon, survivin and livin (ML-IAP)[1]. IAPs represent the last line of defence against inadvertent cell death signalling by inhibiting caspases and by regulating the pro-survival and apoptosis signalling via death receptor complexes.

Caspases are cysteine-dependent aspartyl-specific proteases and central players in cellular apoptosis. They are tightly regulated on several levels. Caspases are expressed as inactive zymogens (procaspases) and their activation follows a strictly controlled pattern. Caspases can be activated by two distinct pathways: the extrinsic apoptotic pathway triggered by external death ligands and the intrinsic apoptotic pathway that is initiated through effects on the mitochondria.

The extrinsic apoptotic pathway is activated when members of the family of TNF-like death ligands, including TNFα, FasL or Apo2L/TRAIL (Apo2L/tumour-necrosis-factor-related apoptosis-inducing ligand), bind to their respective receptors TNFR1, TNFR2, Fas, DR4 or DR5, 'death receptors' located on the cell membrane. When cIAP1 and cIAP2 are absent in the death receptor complex, the binding of death ligands to their receptors leads to the formation of the death-inducing signalling complex (DISC) in which adaptor proteins (FADD and/or TRADD) bind with their death domain and induce the recruitment and activation of the initiator caspases, caspase-8 or -10. More recently it has been shown that loss of cIAP1 and cIAP2 alone can lead to caspase-8 activation in the presence of Smac mimetic through the formation of the ripoptosome composed of RIPK1, FADD, caspase-8 or -10 and cFLIP[2,3]. The activation of caspase-8 or -10 leads to cleavage and activation of the downstream executioner caspases, caspase-3 and -7. These executioner caspases usually remain in an inactive state in the cytosol of the cells. The activation of the executioner caspases eventually results in apoptosis when not inhibited by XIAP[2]. Caspase-8 can also cleave the pro-apoptotic BH3-only protein Bid to truncated Bid (tBID) which can translocate into the mitochondria triggering the activation of the intrinsic pathway resulting in cytochrome c release and apoptosome formation leading to caspase-9 and -3 activation[4].

The intrinsic pathway is activated by 'intrinsic' cell death signals such as hypoxia, genotoxic stress or other types of cellular stress. These signals lead to mitochondrial outer membrane permeabilisation (MOMP) and the release of several proteins into the cytosol including cytochrome-c,

Smac/DIABLO (subsequently referred to as Smac) and others. The assembled multiprotein complex, the apoptosome, induces the activation of caspase-9. Both pathways converge in a common pathway that ultimately leads to apoptosis by inducing the executioner caspases, caspase-3 and -7, which, when not blocked by XIAP, result in DNA fragmentation and cell death[5].

In addition to the controlled activation of caspases, apoptosis is also regulated by IAP inhibition of activated caspases. IAPs regulate the activity of initiator and executioner caspases and are defined by the baculovirus IAP repeat (BIR) domains of approximately 70 to 80 amino acid residues that chelate zinc ions and mediate protein-protein interactions. Of the eight mammalian IAPs that have been identified, four are involved in regulating apoptosis: cIAP1, cIAP2, XIAP and ML-IAP. With the exception of ML-IAP with only one BIR domain, each contains three BIR domains at their amino-terminus, BIR1, BIR2 and BIR3. All four IAPs contain a carboxy-terminal RING (really interesting new gene) zinc-finger domain which has E3 ubiquitin ligase activity. XIAP is the only IAP that inhibits the executioner caspases, caspase-3 and -7 and the initiator caspase-9[6,1].

IAPs and NF-κB pathways – cell survival mechanism

cIAP1and cIAP2 play an important role in regulating TNFα-mediated NF-κB signal transduction via the canonical and non-canonical NF-κB pathways. cIAPs interact with TNFα receptor-associated factor 2 (TRAF2) and activate the canonical pathway through ubiquitylation of receptor-interacting protein (RIP) kinase (RIP1/RIPK1). The non-canonical pathway is inhibited by the cIAPs, TRAF2 and TRAF3 via ubiquitylation and degradation of NF-κB-inducing kinase (NIK). The activated canonical pathway as well the inactivated non-canonical NF-κB pathways influence the expression pattern of a variety of transcription factors that participate in immune responses, inflammation, cell growth, survival and development[1]. The mammalian NF-κB family includes five members that form various transcription regulating complexes. Importantly,

Continued from page 38

10 Du, C, Fang, M, Li, Y, Li, L, Wang, X. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell. 2000 Jul 7;102(1):33-42.
11 Verhagen, AM, Ekert, PG, Pakusch, M, Silke, J, Connolly, LM, Reid, GE, Moritz, RL, Simpson, RJ, Vaux, DL. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell. 2000 Jul 7;102(1):43-53.
12 Chai, J, Du, C, Wu, JW, Kyin, S, Wang, X, Shi, Y. Structural and biochemical basis of apoptotic activation by Smac/DIABLO. Nature. 2000 Aug 24;406(6798):855-62.

Continued on page 40

Continued from page 39

13 Chen, DJ, Huerta, S. Smac mimetics as new cancer therapeutics. Anticancer Drugs. 2009 Sep;20(8):646-58.
14 Tamm, I, Kornblau, SM, Segall, H, Krajewski, S, Welsh, K, Kitada, S, Scudiero, DA, Tudor, G, Qui, YH, Monks, A, Andreeff, M, Reed, JC. Expression and prognostic significance of IAP-family genes in human cancers and myeloid leukemias. Clin Cancer Res. 2000 May;6(5):1796-803.
15 Mizutani, Y, Nakanishi, H, Yamamoto, K, Li, YN, Matsubara, H, Mikami, K, Okihara, K, Kawauchi, A, Bonavida, B, Miki, T. Downregulation of Smac/DIABLO expression in renal cell carcinoma and its prognostic significance. J Clin Oncol. 2005 Jan 20;23(3):448-54. Epub 2004 Nov 30.
16 Mizutani, Y, Nakanishi, H, Yamamoto, K, Li, YN, Matsubara, H, Mikami, K, Okihara, K, Kawauchi, A, Bonavida, B, Miki, T. Downregulation of Smac/DIABLO expression in renal cell carcinoma and its prognostic significance. J Clin Oncol. 2005 Jan 20;23(3):448-54. Epub 2004 Nov 30.
17 Kempkensteffen, C, Jäger, T, Bub, J, Weikert, S, Hinz, S, Christoph, F, Krause, H, Schostak, M, Miller, K, Schrader, M. The equilibrium of XIAP and Smac/DIABLO expression is gradually deranged during the development and progression of testicular germ cell tumours. Int J Androl. 2007 Oct;30(5):476-83. Epub 2007 Feb 12.
18 Bao, ST, Gui, SQ, Lin, MS. Relationship between expression of Smac and Survivin and apoptosis of primary hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int. 2006 Nov;5(4):580-3.

Continued on page 41

the constitutive activation of NF-κB and chronic inflammation play a major role in tumour development, particularly lymphomas, leukaemias and many solid tumours[7-9].

The pro-apoptotic mechanism of Smac

Smac – endogenous IAP antagonist and caspase activator

Smac (second mitochondria-derived activator of caspase), also known as DIABLO (direct inhibitor of apoptosis protein-binding protein with low pI), is a pro-apoptotic mitochondrial protein that is released upon apoptotic stimuli into the cytosol and binds to the IAPs[10,11]. Smac antagonises IAP-mediated caspase inhibition by direct inhibition and/or induces proteasomal degradation of some members of the IAP family (cIAP1 and cIAP2). A study published in 2000 by Yigong Shi's laboratory at Princeton University described the structural and biochemical basis of apoptotic activation by Smac[12]. Smac homodimerises and forms a stable protein dimer. The ability of SMAC to promote both the proteolytic activation of pro-caspase-3 and the enzymatic activity of mature caspase-3 depends on its ability to specifically interact with XIAP. Smac binds to the BIR1/BIR2 linker region and BIR-3 of XIAP disrupting the inhibition of caspase-3 and -7 and preventing cell death[1,13].

Smac and Smac mimetics induce proteasomal degradation of cIAP1 and cIAP2 resulting in inhibition of the canonical pathway and activation of the non-canonical pathway via NIK stabilisation.

IAPs, Smac and cancer

Increased expression of IAPs is found in many types of human cancers and is associated with chemoresistance, disease progression and poor prognosis. *In vitro*, higher expression of IAPs promotes resistance to chemotherapy agents as well as radiation. In acute myeloid leukaemia, higher expression of XIAP may lead to a poorer prognosis[14]. IAPs protect tumour cells from the cytotoxic effects of cancer-related inflammation induced by TNFα or TRAIL that are prevalent in the tumour microenvironment of many tumours. Therefore, a therapeutic approach that results in the degradation of cIAPs and/or antagonism of XIAP could promote the lethal effects of TNFα on cancer cells while shutting down the survival pathway mediated by TNFα driven NF-κB expression.

Preclinical studies have shown that Smac expression can be an important factor in determining a cancer cell's sensitivity to undergo apoptosis when induced by a variety of apoptotic stimuli. Several studies have shown that reduced expression of Smac is associated with cancer progression. For example, Mizutani et al showed that Smac expression was downregulated in renal cell carcinoma (RCC), and an absence of Smac protein expression predicted worse outcomes in RCC[15]. Another study showed that RCC patients with low Smac mRNA expression had a four-times higher risk of dying from RCC than those with high expression[16,17]. Additionally, testicular tumours with a more advanced malignant phenotype showed lower Smac mRNA expression levels. The mRNA as well as protein expression was reduced in hepatocellular carcinoma (HCC) when compared to normal hepatic tissue[18].

The fact that overexpression of Smac sensitises neoplastic cells to apoptosis[19], together with the importance of the role played by Smac and IAPs in regulating apoptosis in tumour cells, became the rationale for the design and development of Smac mimetics as a new class of cancer therapeutics.

Smac mimetics – a novel class of therapeutics regulating apoptosis and NF-κB

The discovery of Smac mimetics or IAP antagonists was enabled by the elucidation of the crystal structure of the interaction between Smac and IAPs[12]. Smac mimetics facilitate apoptotic cell death in tumour cells through multiple mechanisms: they bind and antagonise IAPs and reactivate the apoptotic pathway; Smac mimetics can eliminate IAPs by promoting autoubiquitylation and proteasomal degradation of cIAPs; and they activate the cell's extrinsic apoptotic pathway by autocrine TNFα stimulation or when TNFα or TRAIL are present in the tumour microenvironment. Interestingly, the induction of apoptosis is highly specific for susceptible tumours and spares normal tissue. In tissue culture, Smac mimetics are capable of killing tumour cells in the picomolar concentration range while having no effect on normal cells in the 100 micromolar range. This marked selectivity is thought to be a result of elevated levels of key caspases in tumour cells.

Several Smac mimetics have been developed that have demonstrated good anti-tumour activity in preclinical studies. Bivalent Smac mimetics are significantly more potent than monovalent Smac mimetics generally have improved pharmacokinetics. Bivalent mimetics have a higher molecular weight and may require a parenteral administration[13].

Of those Smac mimetics that have entered the clinic, the most advanced is TL32711, a potent bivalent small molecule Smac mimetic. Preclinical studies in patient-derived tumour xenotransplant

models in mice have shown that TL32711 leads to tumour-regression as a single agent and that it displays synergy when combined with specific chemotherapies. TL32711 has been shown to restore cancer cell sensitivity to apoptotic stimuli, such as TNFα or TRAIL, in a panel of patient-derived human cell lines.

A Phase I first-in-man study of TL32711 in adult patients with advanced solid tumours and lymphoma showed that intravenous TL32711 was well tolerated with no dose-limiting toxicities (maximum tolerated dose had not been reached) and demonstrated evidence of anti-tumour activity[20]. Pharmacokinetics were dose proportional with moderate to low inter-patient variability in Cmax and AUC and a long terminal half-life in plasma. Importantly, TL32711 showed high concentrations and good retention in tumour tissues with suppression of the cIAP1 target for the entire one-week dose interval. TL32711 also induced activation of serum caspase-3 and -7. A Phase Ib/IIa five-arm study of TL32711 in combination with five different chemotherapies is currently under way. Additional Phase I and II clinical studies are planned for both solid tumours and hematological malignancies.

Other Smac mimetics in clinical development include:

LCL161(Novartis) – LCL161 is a high affinity, monovalent Smac mimetic that has been tested as both single agent and in combination with other agents showing good anti-tumour activity in solid tumours including triple-negative breast cancer[21]. LCL-161 has completed a Phase I safety and efficacy study and is currently being evaluated in Phase I study in advanced solid tumours in combination with paclitaxel[22,23].

GDC-0917 (Genentech) – GDC-0197 is a monovalent Smac mimetic that is currently in Phase I study evaluating safety, tolerability and pharmacokinetics in patients with refractory solid tumours or lymphoma[24].

HGS1029 (Human Genome Sciences) – A bivalent Smac mimetic, in preclinical studies HGS1029 showed good anti-tumour activity against a number of tumour types alone and in combination with other agents. HGS1029 is currently in Phase I study evaluating safety and tolerability as monotherapy in patients with advanced solid tumours[25]. HGSI also plans to study HGS1029 in combination with human monoclonal TRAIL receptor antibodies.

AT-406 (Ascenta) – AT-406 is a monovalent Smac mimetic that has shown good anti-tumour activity against xenographs of a number of tumour types. AT-406 is currently being evaluated in Phase I study as a single agent in patients with advanced solid tumours and lymphomas and in combination with daunorubicin and cytarabine in patients with poor-risk acute myelogenous leukaemia (AML)[26,27].

Conclusion

Smac mimetics are a new class of cancer therapeutics with great potential to overcome the limitations of current anticancer therapies. Smac mimetics are generally well tolerated and have demonstrated rapid suppression of their target (the IAPs), activation of apoptosis and anti-tumour activity. Additionally, Smac mimetics may help to overcome the resistance associated with conventional cancer therapies mediated by the NF-κB and IAP pathways. DDW

Dr Mark A. McKinlay *is Co-Founder, Senior Vice-President, Research & Development and Chief Scientific Officer of TetraLogic Pharmaceuticals. Prior to TetraLogic, Dr McKinlay was a co-founder and Vice-President of R&D ViroPharma Corporation. Dr McKinlay was also a Senior Director of Virology and Oncopharmacology at Sterling Winthrop Pharmaceuticals Research Division. He was a post-doctoral fellow at the Johns Hopkins School of Public Health and holds a PhD and MS in biology from Rennselaer Polytechnic Institute.*

Continued from page 40

19 Martinez-Ruiz, G, Maldonado, V, Ceballos-Cancino, G, Grajeda, JP, Melendez-Zajgla, J. Role of Smac/DIABLO in cancer progression. J Exp Clin Cancer Res. 2008 Sep 26;27:48.
20 Amaravadi, RK, Schilder, RJ, Dy, GK, Ma, WW, Fetterly, GJ, Weng, DE, Graham, MA, Burns, JM, Chunduru, SK, Condon, SM, McKinlay, MA, Adjei, AA. Phase 1 study of the Smac mimetic TL32711 in adult subjects with advanced solid tumors & lymphoma to evaluate safety, pharmacokinetics, pharmacodynamics and anti-tumor activity. Poster abstract #2532. 102nd AACR annual meeting. 2011, Orlando, FL.
21 The Pipeline of Novartis Oncology, Novartis website, Novartis, 2011.
22 Safety and Efficacy of LCL161 in Patients With Solid Tumors. Clinicaltrials.gov, 2011.
23 A Study of LCL161 in Combination With Weekly Paclitaxel in Adult Patients With Advanced Solid Tumors. Clinicaltrials.gov, 2011.
24 A Study Evaluating the Safety, Tolerability and Pharmacokinetics of GDC-0917 Administered to Patients With Refractory Solid Tumors or Lymphoma . Clinicaltrials.gov, 2011.
25 A Study of HGS1029 (AEG40826-2HCl) in Subjects With Advanced Solid Tumors. Clinicaltrials.gov, 2011.
26 Dose Escalation Study of Safety and Tolerability of AT-406 in Patients With Advanced Solid Tumors and Lymphomas. Clinicaltrials.gov, 2011.
27 Study of the Safety, Tolerability, Pharmacokinetics and Pharmacodynamic Properties of Oral AT-406 in Combination With Daunorubicin and Cytarabine in Patients With Poor-risk Acute Myelogenous Leukemia (AML). Clinicaltrials.gov, 2011.