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Wang et al.

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(54) **EPIGENETIC MODIFICATIONS AT TNF α AS BIOMARKERS FOR SENSITIVITY TO SMAC MIMETIC-INDUCED APOPTOSIS**

(58) **Field of Classification Search** None
See application file for complete search history.

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(56) **References Cited**

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PUBLICATIONS

Li, et al., Science vol. 305, pp. 1471-1474 (2004).*

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 472 days.

* cited by examiner

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(57) **ABSTRACT**

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The invention provides a method for determining sensitivity of a cell population to apoptosis induced by Smac mimetics as a single agent or in combination with other chemodrugs, which method comprises assaying for an epigenetic modification at the TNF α locus in the cell population as compared to the TNF α locus in a cell population not sensitive to apoptosis induced by Smac mimetics as a single agent or in combination with other chemodrugs, wherein the presence of the epigenetic modification indicates that the cell population is sensitive to apoptosis induced by Smac mimetics as a single agent or in combination with other chemodrugs.

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C12Q 1/68 (2006.01)

(52) **U.S. Cl.**
USPC **435/6.13; 435/377; 514/2.3**

20 Claims, 7 Drawing Sheets

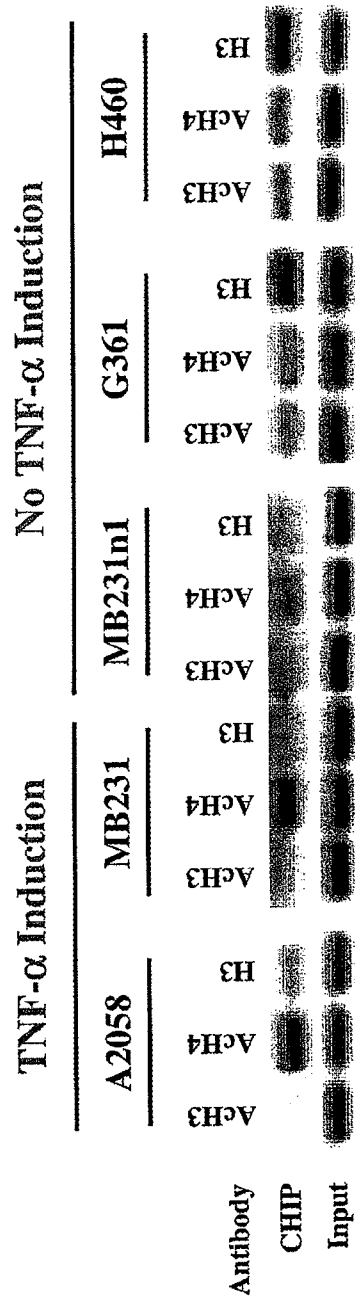


Figure 1

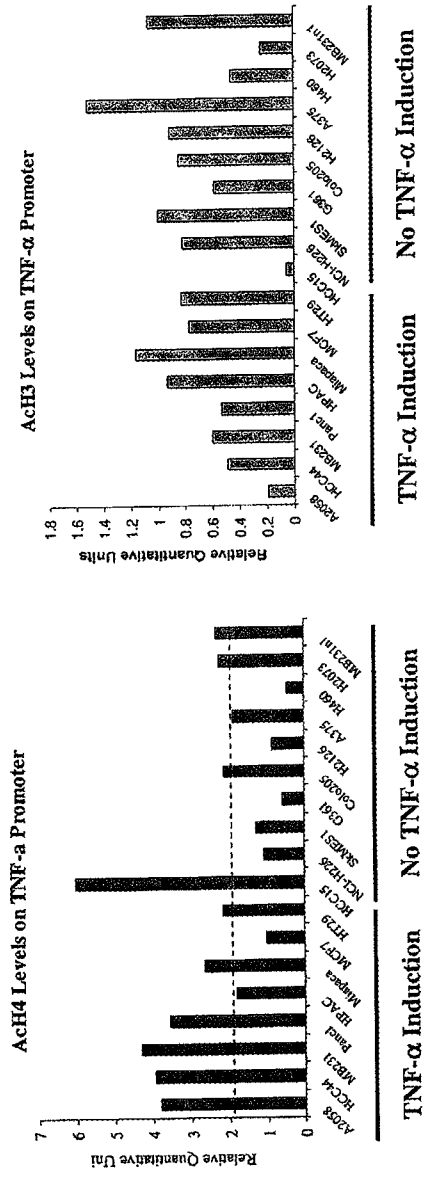


Figure 2

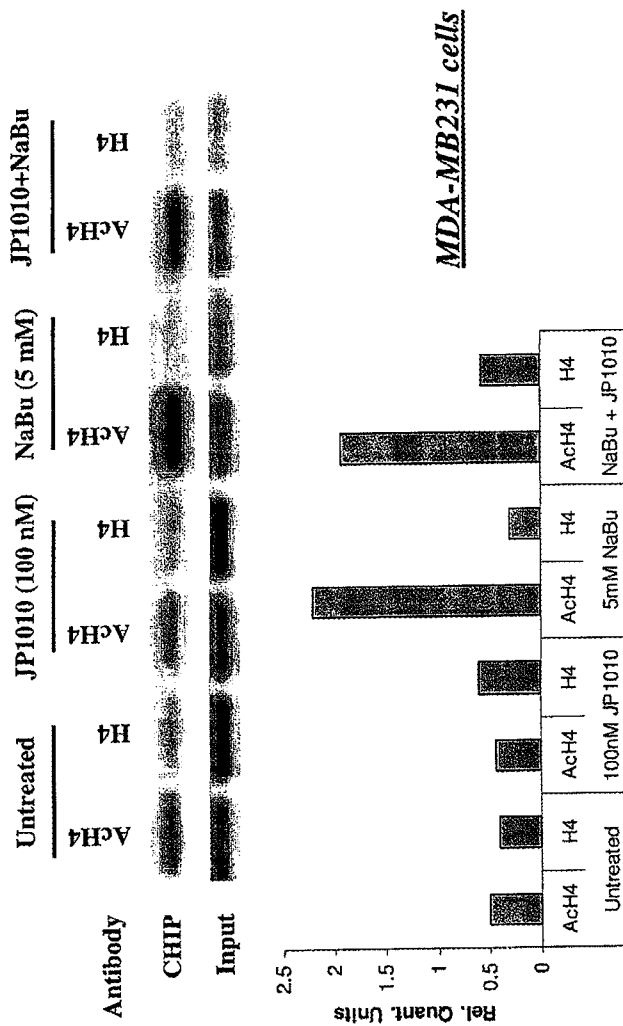


Figure 3

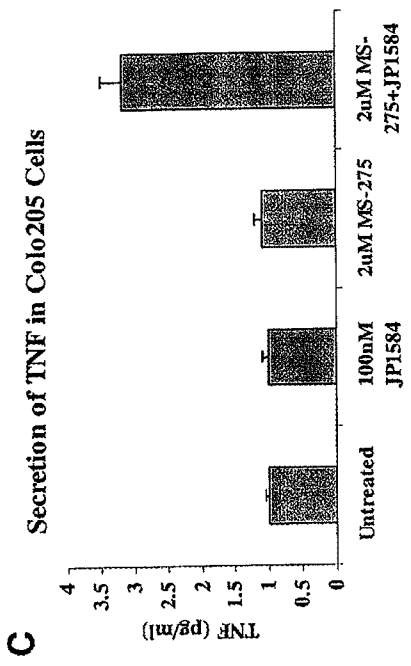
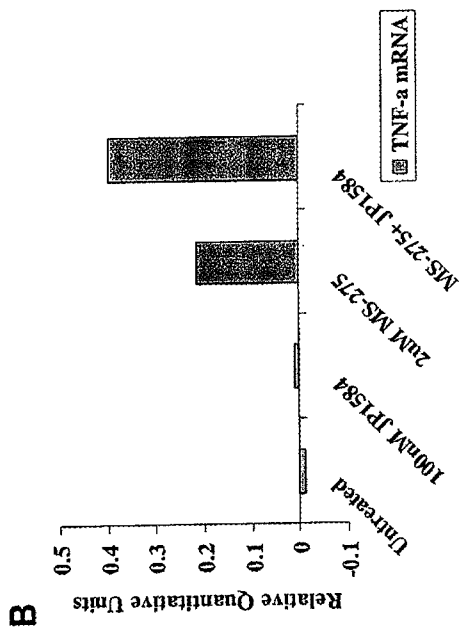
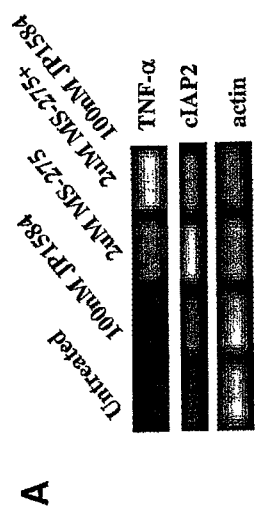


Figure 4

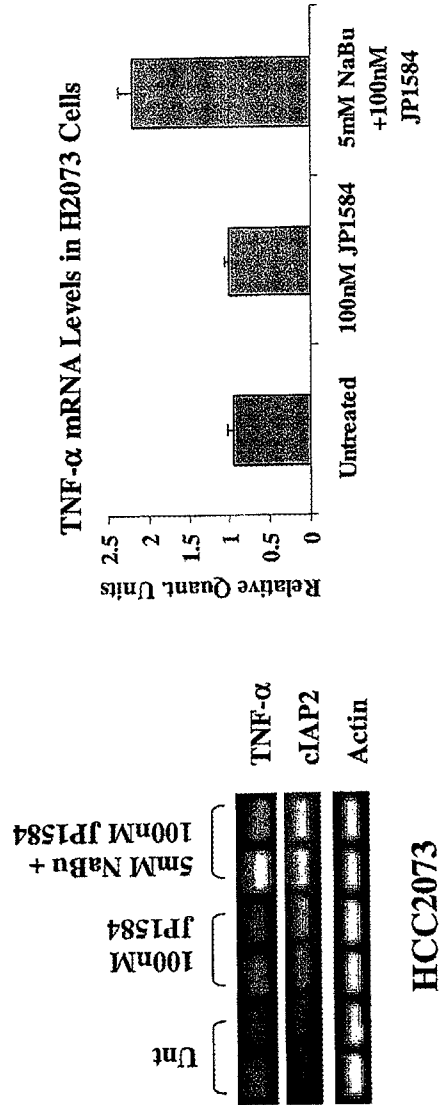


Figure 5



Figure 6

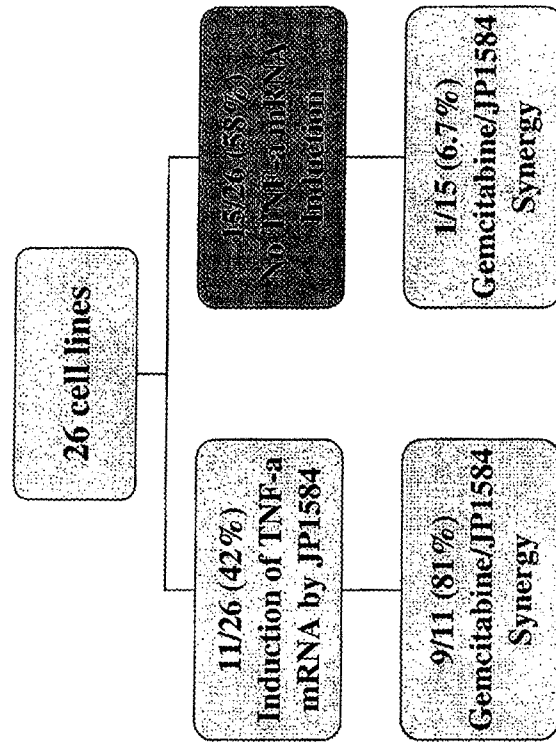


Figure 7

EPIGENETIC MODIFICATIONS AT TNF α AS BIOMARKERS FOR SENSITIVITY TO SMAC MIMETIC-INDUCED APOPTOSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a non-provisional of U.S. Application Ser. No. 61/168,191 filed on Apr. 9, 2009. The contents of the application listed in this paragraph are fully incorporated by reference herein.

TECHNICAL FIELD

The field of the invention is biomarkers for sensitivity to Smac mimetic-induced apoptosis. The biomarkers of the invention include epigenetic modifications at the TNF α locus. These biomarkers are useful for predicting the sensitivity of the cell population to apoptosis induced by Smac mimetics (as a single agent or in combination with other chemodrugs).

BACKGROUND ART

Apoptosis plays a central role in the development and homeostasis of all multi-cellular organisms. Abnormal inhibition of apoptosis is a hallmark of cancer and autoimmune diseases, whereas excessive activation of cell death is implicated in neuro-degenerative disorders such as Alzheimer's disease. Pro-apoptotic chemotherapeutic drugs provide a recent approach to overcoming the clinical problem of drug resistance. See, e.g., Makin, et al., *Cell Tissue Res.* 301:143-52 (2000).

The mechanism of apoptosis is conserved across species and executed with a cascade of sequential activation of proteases called caspases. Once activated, these caspases are responsible for proteolytic cleavage of a broad spectrum of cellular targets that ultimately lead to cell death. IAPs (inhibitor-of-apoptosis proteins) regulate apoptosis by inhibiting caspases; and a protein called Smac ("Smac" stands for second mitochondria-derived activator of caspases, and is a mitochondrial protein) binds to and inhibits IAPs, and thereby promotes caspase activation.

Defective apoptosis regulation can confer resistance to many current treatment protocols, leading to tumor growth. This may occur as a result of overexpression of IAPs, which inhibit the caspases that would otherwise initiate apoptosis. Alternatively, deregulation can occur as a result of underproduction of the Smac peptides that act to inhibit IAP activity. Deficiency of Smac can thus allow IAP to prevent apoptosis from occurring when it should, and a Smac mimetic like the present compounds can replace the activity of Smac and thus promote desired apoptosis.

Debatin, et al., WO 03/086470, describes Smac-peptides as therapeutic agents useful against cancer and autoimmune diseases; they are reported to act by sensitizing the cells toward TRAIL-induced or anticancer drug-induced apoptosis. (TRAIL stands for TNF α related apoptosis-inducing ligand). See also Li, et al., *Science* 305:1471-1474 (2004). Debatin provides in vivo evidence that Smac induces the eradication of certain tumors such as glioblastoma tumor models in animals when administered in combination with TRAIL. According to Debatin, aggressive cancer phenotypes, which result from deregulation of signaling pathways, commonly fail to undergo apoptosis when they otherwise would, allowing rapid and abnormal tissue growth. Bockbrader, et al., disclose efficacy of Smac mimic compounds on

breast cancer cell lines when used in conjunction with TRAIL or etoposide, or when used in cells that express TRAIL at relatively high levels. *Oncogene* 24:7381-7388 (2005).

Similarly, according to Debatin, defects in apoptosis regulation play a key role in the pathogenesis of autoimmune disorders, including lupus erythematosus disseminatus and rheumatoid arthritis. Accordingly, compounds that mimic the activity of Smac can treat some of the effects of such conditions.

The protein Smac has been shown to inhibit a wide variety of IAPs, and is believed to be a key regulator of apoptosis in mammals. See Du, et al., *Cell* 102:33-43 (2000); Verhagen et al., *Cell* 102:43-53 (2000); and Vucic et al., *Biochem. J.* 385(1):11-20 (2005). N-terminal Smac-derived peptides and mimetics have been shown to similarly inhibit IAPs, and promote caspase-8 activation. See Petersen, et al., *Cancer Cell* 12:445-456 (2007); Varfolomeev, et al., *Cell* 131:669-681 (2007); and Vince, et al., *Cell* 131:682-693 (2007).

These studies further showed that TNF α , a death receptor ligand, is the major actor in cancer cell apoptosis induced by Smac mimetics. IAPs are components of TNFR (tumor necrosis factor receptor), so IAP inhibitors can divert TNFR signaling from an NF- κ B-mediated pro-inflammatory signal, to an anti-inflammatory apoptotic signal. Acute reduction in these IAPs activates the NF- κ B pathways and causes auto-crine TNF α production, which in turn leads to TNFR occupancy, caspase-8 activation, and cell death.

Eukaryotic DNA is packaged into chromatin which appears as a series of "beads on a string," with the beads being the individual nucleosomes. Kornberg & Lorch, *Cell* 98:285-294 (1999). Each nucleosome consists of eight core histone proteins (two each of H3, H4, H2A and H2B), which are wrapped by 147 base pairs of DNA in a left-handed superhelix, forming the intact nucleosome. Luger, et al., *Nature* 389:251-260 (1997).

Several factors, including DNA methylation, histone modifications, and small nuclear RNAs, have been implicated in the regulation of transcription from chromatin, and are referred to as "epigenetic regulations." Epigenetic mechanisms are essential for development, cell differentiation, and protection against viral genomes, and seem to be critical for the integration of endogenous and environmental signals during the life of a cell or an organism. By analogy, deregulation of epigenetic mechanisms has been associated with a variety of human diseases, most notably cancer.

Different types of epigenetic modifications are closely linked and often act in self-reinforcing manner in the regulation of different cellular processes. DNA methylation and histone acetylation are major epigenetic modifications that are dynamically linked in the epigenetic control of gene expression and their deregulation plays an important role in tumorigenesis. See Feinberg, et al., *Nat. Rev. Genet.* 7:21-33 (2006); Jones & Baylin, *Nat. Rev. Genet.* 3:415-428 (2002). Recent studies suggested that an intimate communication and mutual dependence exists between histone acetylation and DNA methylation in the process of gene silencing. Communication between histone acetylation and cytosine methylation may proceed in both directions. In one scenario, DNA methylation may be the primary mark for gene silencing that triggers events leading to non-permissive chromatin state. In another scenario, the loss of histone acetylation may serve as the initial event of gene silencing, which is followed by DNA methylase targeting and induction of local DNA hypermethylation. See Vaissiere, et al., *Mut. Res.* 659:40-48 (2008).

DISCLOSURE OF THE INVENTION

Provided herein are methods useful for determining the sensitivity of a cell population to apoptosis induced by Smac

mimetics (as a single agent or in combination with other chemodrugs) by analyzing the epigenetic status of the TNF α promoter. Both DNA-methylation and histone modifications may play important roles in the epigenetic regulation of TNF α expression and may correlate with sensitivity of a cell population to apoptosis induced by Smac mimetics (as a single agent or in combination with other chemodrugs). Thus, these epigenetic events at the TNF α promoter could serve as biomarkers for sensitivity of tumor cells to chemotherapeutic treatments such as Smac mimetics, as a single agent or in combination with other chemodrugs, as described hereafter.

Thus, in one aspect, provided herein is a method for determining the sensitivity of a cell population to apoptosis induced by Smac mimetics (as a single agent or in combination with other chemodrugs), which method comprises assaying for an epigenetic alteration at the TNF α locus in the cell population, wherein the presence/absence of the epigenetic modification indicates that the cell population is sensitive to apoptosis induced by Smac mimetics (as a single agent or in combination with other chemodrugs). Without being bound by theory, the present method is believed to base on the finding that such epigenetic modification leads to TNF α expression induced by a Smac mimetic, thus making the cell population sensitive to apoptosis induced by Smac mimetics (as a single agent or in combination with other chemodrugs).

Also provided herein is a method for sensitizing a cell population to apoptosis induced by Smac mimetics (as a single agent or in combination with other chemodrugs), which method comprises treating the cell population with an agent which alters an epigenetic modification at the TNF α locus.

Further provided herein is a method of inducing apoptosis in a cell population, which method comprises: a) determining the sensitivity to apoptosis induced by Smac mimetics (as a single agent or in combination with other chemodrugs) of the cell population by assaying for an epigenetic alteration at the TNF α locus in the cell population; and b) treating the cell population with an apoptosis-inducing agent comprising a Smac mimetic if the cell population is sensitive to Smac mimetic-induced apoptosis.

Still further provided herein is a method of inducing apoptosis in a cell population, which method comprises treating the cell population with an apoptosis-inducing agent comprising a Smac mimetic and an agent that changes an epigenetic modification at the TNF α locus.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Acetyl-H4 levels at the TNF α locus correlate with Smac mimetic-induced TNF α expression.

FIG. 2. Higher levels of acetylated H4 at the TNF α locus correlate with Smac mimetic-induced TNF α expression.

FIG. 3. Smac mimetics do not affect acetylated Histone H4 levels at the TNF α locus.

FIG. 4. HDAC inhibitor MS-275 Primes Colo205 cells for Smac mimetic-induced TNF α production.

FIG. 5. Sodium butyrate (NaBu), an HDAC inhibitor, increases Smac mimetic-induced TNF α production in H2073 cells.

FIG. 6. Smac mimetics do not induce TNF α production in resistant cells.

FIG. 7. Correlation between induced TNF α expression and Gemcitabine/IP1584 synergy.

DETAILED DESCRIPTION OF THE INVENTION

Smac mimetics have been used as a new generation of apoptosis-inducing agents in killing cancer cells by antago-

nizing the activity of IAPs such as XIAP, cIAP1, and cIAP2. However, there remains a need for a treatment of cells that are resistant to apoptosis induced by Smac mimetics, as a single agent or in combination with other chemodrugs. The resistance phenotype may be related to the lack of TNF α expression in response to Smac mimetic treatment in these cell populations. Activation of the NF- κ B signaling pathway leads to the induction of many genes. Sensitive cell lines already secrete TNF α before any treatment (Petersen, et al., *Cancer Cell* 12:445-456 (2007)), although in most cases TNF α secretion is further increased in these same cell lines upon treatment with Smac mimetics.

Thus, cancer cells can generally be defined by their resistance or sensitivity to Smac mimetic-induced apoptosis. One type of cancer cells is sensitive to Smac mimetic-induced apoptosis. In this type of cells, Smac mimetics induce or enhance TNF α synthesis and secretion and render the cells sensitive to TNF α -induced apoptosis by forming a death domain kinase RIP1-dependent caspase-8-activating complex. Another type of cancer cells does not respond to Smac mimetic treatment. In these cells, treatment with a Smac mimetic fails to induce TNF α secretion. See Wu, et al., *Cell* 131:655-658 (2007).

The methods provided herein are useful for distinguishing these two types of cancer cells by analyzing the epigenetic alterations at the TNF α locus in such cells. Certain epigenetic alterations, such as acetylation of histone H4, were found to correlate with sensitivity of such cell population to Smac mimetic-induced apoptosis.

The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the Examples included herein. It is to be understood that the terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting.

DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. All patents, patent applications (published or unpublished), and other publications referred to herein are incorporated by reference in their entirety. If a definition set forth in this section is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth in this section prevails over the definition that is incorporated herein by reference.

As used herein, the singular form "a", "an", and "the" include plural references unless indicated otherwise. For example, "a" dimer includes one of more dimers.

A "Smac mimetic" is a molecule that has the function of inhibiting the activity of IAP proteins by mimicking the activity of the Smac N-terminal AVPI peptide. Smac mimetics have been described; see Fesik, et al., WO 2002030959; McLendon, et al., WO 2002096930; Shi, WO 2002026775; Debatin, et al., WO 2003086470; Alnemri, WO 2003010184; U.S. Pat. Publ. No. 20020132786; U.S. Pat. Publ. No. 20020160975; U.S. Pat. Publ. No. 20020177557; U.S. Pat. No. 6,608,026; WO 2006091972; WO 2005084317; Harran, et al., U.S. Pat. No. 7,309,792; WO 2008014263; WO 2008014252 The monovalent smac mimetics are designed to mimic the binding of a single AVPI binding motif to IAP proteins, whereas the bivalent compounds contain two AVPI binding motif mimetics tethered together through a linker.

Studies from several groups have clearly demonstrated that both monovalent and bivalent Smac mimetics not only enhance the antitumor activity of other anticancer agents but also can induce apoptosis as single agents in a subset of human cancer cell lines in vitro and are capable of achieving tumor regression in animal models of human cancer. In general, bivalent Smac mimetics are 100-1000 times more potent than their corresponding monovalent Smac mimetics in induction of apoptosis in tumor cells.

As used herein, an "epigenetic modification" is an inheritable change in gene function that occurs without a change in the DNA sequence. Epigenetic modifications include DNA methylation, histone modification (e.g., acetylation), and RNA interference, etc. In addition, "epigenetic modifications" as used herein may also include chromosomal binding of proteins that are responsible for DNA methylation, histone modification (e.g., acetylation), and RNA interference, etc., as well as proteins that binds to modified histones or methylated DNA.

As used herein, the "TNF α locus" refers to the genomic sequence which includes the promoter, the exons, the introns, and the intergenic regions both upstream and downstream of the TNF α gene.

The terms "sensitize" and "sensitizing," as used herein, refer to making, through the administration of a first agent (e.g., a histone deacetylase (HDAC) inhibitor), a cell population more susceptible, or more responsive, to the biological effects (e.g., promotion or retardation of an aspect of cellular function including, but not limited to, cell division, cell growth, proliferation, invasion, angiogenesis, or apoptosis) of a second agent. The sensitizing effect of a first agent on a target cell can be measured as the difference in the intended biological effect (e.g., promotion or retardation of an aspect of cellular function including, but not limited to, cell growth, proliferation, invasion, angiogenesis, or apoptosis) observed upon the administration of a second agent with and without administration of the first agent. The response of the sensitized cell can be increased by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 100%, at least 150%, at least 200%, at least 350%, at least 300%, at least 350%, at least 400%, at least 450%, or at least 500% over the response in the absence of the first agent.

It is understood that aspects and embodiments of the invention described herein include "consisting" and/or "consisting essentially of" aspects and embodiments.

Other objects, advantages and features of the present invention will become apparent from the following specification taken in conjunction with the accompanying drawings.

MODES OF CARRYING OUT THE INVENTION

One aspect of the invention is a method for determining sensitivity of a cell population to Smac mimetic-induced apoptosis as a single agent or in combination with other

chemodrugs, which method comprises assaying for an epigenetic modification at the TNF α locus in the cell population, wherein the presence/absence of the epigenetic modification indicates that the cell population is sensitive to apoptosis induced by Smac mimetics as a single agent or in combination with other chemodrugs.

The presence/absence of certain epigenetic modifications is determined in relation to a background cell population. For example, a cell population that is known to be sensitive to apoptosis induced by Smac mimetics (as a single agent or in combination with other chemodrugs) may be used as a positive control while a cell population that is known to be resistant may be used as a negative control. Alternatively, a panel of both sensitive and resistant cell populations can be analyzed for such epigenetic modification and a standard curve may be constructed from such survey, and analysis result from a cell population may be compared to such standard curve in order to determine the level of epigenetic modification and sensitivity of such cell population. In the case where a tumor sample from a patient is analyzed, similar tumor samples from other patients that have been shown to be sensitive/resistant to apoptosis induced by Smac mimetics (as a single agent or in combination with other chemodrugs) may be used as positive/negative controls.

Epigenetic Modifications at the TNF α Locus

A major type of epigenetic modification is the post-translational modification of histone proteins. The N-terminal domains of histone proteins H3 and H4 are subject to a variety of modifications, including methylation, acetylation, phosphorylation, ubiquitination, and ADP-ribosylation. Zhang & Reinberg, *Genes Dev.* 15:2343-2360 (2001); Sims, et al., *Trends Genet.* 19:629-639 (2003). These modifications occur especially at lysine, serine, and arginine residues. Research into these modifications has led to the realization that chromatin is not merely a scaffolding structure, but also a dynamic entity capable of regulating gene expression and cellular functions. This has led to the notion of the "histone code," which postulates that the type and number of histone N-terminal tail modifications serve as an epigenetic regulatory mechanism governing specific transcriptional states and biological outcomes Strahl & Allis, *Nature* 403:4145 (2000); Turner, *Cell* 111:285-291 (2002); Jenuwein & Allis, *Science* 293:1074-1079 (2001); Iizuka & Smith, *Curr. Opin. Genet. Dev.* 13:154-160 (2003). Studies supporting the notion of a "histone code" have demonstrated that histone modifications participate in a variety of essential biological processes, including gene activation, gene silencing, gene repression, and X chromosome inactivation. Lachner & Jenuwein, *Curr. Opin. Cell Biol.* 14:286-298 (2002); Lachner, et al., *J. Cell Sci.* 116:2117-2124 (2003). The histone code may be characterized by single histone modifications or by patterns of histone modifications. Berger, *Curr. Opin. Genet. Dev.* 12:142148 (2002).

A list of currently known histone modifications, their proposed functions and responsible enzymes is provided in Table 1:

TABLE 1

Types of Histone Modifications, Sites of Modification, Proposed Functions and Enzymes Involved			
Histone	Site	Histone-modifying Enzymes	Proposed Function
Acetylation			
H2A	Lys4 (<i>S. cerevisiae</i>)	Esa1	transcriptional activation
	Lys5 (mammals)	Tip60, p300/CBP	transcriptional activation
	Lys7 (<i>S. cerevisiae</i>)	Hat1	unknown
		Esa1	transcriptional activation

TABLE 1-continued

Types of Histone Modifications, Sites of Modification, Proposed Functions and Enzymes Involved				
Histone	Site	Histone-modifying Enzymes	Proposed Function	
H2B	Lys5	p300, ATF2	transcriptional activation	
	Lys11 (<i>S. cerevisiae</i>)	Gcn5	transcriptional activation	
	Lys12 (mammals)	p300/CBP, ATF2	transcriptional activation	
	Lys15 (mammals)	p300/CBP, ATF2	transcriptional activation	
	Lys16 (<i>S. cerevisiae</i>)	Gcn5, Esa1	transcriptional activation	
H3	Lys20	p300	transcriptional activation	
	Lys4 (<i>S. cerevisiae</i>)	Esa1	transcriptional activation	
		Hpa2	unknown	
	Lys9	unknown	histone deposition	
		Gcn5, SRC-1	transcriptional activation	
	Lys14	unknown	histone deposition	
		Gcn5, PCAF	transcriptional activation	
		Esa1, Tip60	transcriptional activation	
			DNA repair	
		SRC-1	transcriptional activation	
		Elp3	transcriptional activation (elongation)	
			unknown	
		Hpa2	RNA polymerase III transcription	
		hTFIIIC90	RNA polymerase II transcription	
		TAF1	RNA polymerase II transcription	
		Sas2	euchromatin	
		Sas3	transcriptional activation (elongation)	
			transcriptional activation	
		Lys18	p300 Gcn5	transcriptional activation, DNA repair
			p300/CBP	DNA replication, transcriptional activation
	Lys23	unknown Gcn5	histone deposition, transcriptional activation, DNA repair	
		Sas3	transcriptional activation (elongation)	
	Lys27	p300/CBP Gcn5	transcriptional activation	
	Lys56 (<i>S. cerevisiae</i>)	Spt10	transcriptional activation, DNA repair	
H4	Lys5	Hat1 Esa1, Tip60	histone deposition, transcriptional activation, DNA repair	
		ATF2 Hpa2	transcriptional activation, unknown	
	Lys8	p300 Gcn5, PCAF Esa1, Tip60	transcriptional activation, transcriptional activation, transcriptional activation, DNA repair	
		ATF2 Elp3	transcriptional activation, transcriptional activation (elongation)	
	Lys12	p300 Hat1	transcriptional activation, histone deposition	
		Esa1, Tip60	telomeric silencing, transcriptional activation, DNA repair	
	Lys16	Hpa2 p300 Gcn5 MOF (<i>D. melanogaster</i>) Esa1, Tip60	unknown, transcriptional activation, transcriptional activation, transcriptional activation, DNA repair	
		ATF2 Sas2	transcriptional activation, euchromatin	
	Lys91 (<i>S. cerevisiae</i>)	Hat1/Hat2	chromatin assembly	
	Methylation			
	H1	Lys26	Ezh2	transcriptional silencing
	H3	Lys4	Set1 (<i>S. cerevisiae</i>)	permissive euchromatin (di-Me)
		Set7/9 (vertebrates)	transcriptional activation (tri-Me)	
		MLL, ALL-1	transcriptional activation	
		Ash1 (<i>D. melanogaster</i>)	transcriptional activation	

TABLE 1-continued

Types of Histone Modifications, Sites of Modification, Proposed Functions and Enzymes Involved			
Histone	Site	Histone-modifying Enzymes	Proposed Function
	Arg8	PRMT5	transcriptional repression
	Lys9	Suv39h,Clr4	transcriptional silencing (tri-Me)
		G9a	transcriptional repression
		SETDB1	genomic imprinting
		Dim-5 (<i>N. crassa</i>), Kryptonite (<i>A. thaliana</i>)	transcriptional repression (tri-Me)
		Ash1 (<i>D. melanogaster</i>)	DNA methylation (tri-Me)
	Arg17	CARM1	transcriptional activation
	Lys27	Ezh2	transcriptional silencing
		G9a	X inactivation (tri-Me)
	Lys36	Set2	transcriptional silencing
	Lys79	Dot1	transcriptional activation (elongation)
			euchromatin
			transcriptional activation (elongation)
			checkpoint response
H4	Arg3	PRMT1	transcriptional activation
		PRMT5	transcriptional repression
	Lys20	PR-Set7	transcriptional silencing (mono-Me)
		Suv4-20h	heterochromatin (tri-Me)
		Ash1 (<i>D. melanogaster</i>)	transcriptional activation
		Set9 (<i>S. pombe</i>)	checkpoint response
	Lys59	unknown	transcriptional silencing
		Phosphorylation	
H1	Ser27	unknown	transcriptional activation, chromatin decondensation
H2A	Ser1	unknown	mitosis, chromatin assembly
		MSK1	transcriptional repression
	Thr119 (<i>D. melanogaster</i>)	NHK1	mitosis
	Ser122 (<i>S. cerevisiae</i>)	unknown	DNA repair
	Ser129 (<i>S. cerevisiae</i>)	Mec1, Tel1	DNA repair
	Ser139 (mammalian H2AX)	ATR, ATM, DNA-PK	DNA repair
H2B	Ser10 (<i>S. cerevisiae</i>)	Ste20	apoptosis
	Ser14 (vertebrates)	Mst1	apoptosis
		unknown	DNA repair
	Ser33 (<i>D. melanogaster</i>)	TAF1	transcriptional activation
H3	Thr3	Haspin/Gsg2	mitosis
	Ser10	Aurora-B kinase	mitosis, meiosis
		MSK1, MSK2	immediate-early gene activation
		IKK- α	transcriptional activation
		Snf1	transcriptional activation
	Thr11 (mammals)	Dlk/Zip	mitosis
	Ser28 (mammals)	Aurora-B kinase	mitosis
		MSK1, MSK2	immediate-early activation
H4	Ser1	unknown	mitosis, chromatin assembly
		CK2	DNA repair
		Ubiquitylation	
H2A	Lys119 (mammals)	Ring2	spermatogenesis
H2B	Lys120 (mammals)	UbcH6	meiosis
	Lys123 (<i>S. cerevisiae</i>)	Rad6	transcriptional activation
			euchromatin
		Sumoylation	
H2A	Lys126 (<i>S. cerevisiae</i>)	Ubc9	transcriptional repression
H2B	Lys6 or Lys7 (<i>S. cerevisiae</i>)	Ubc9	transcriptional repression
H4	N-terminal tail (<i>S. cerevisiae</i>)	Ubc9	transcriptional repression
		Biotinylation	
H2A	Lys9	biotinidase	unknown
	Lys13	biotinidase	unknown
H3	Lys4	biotinidase	gene expression
	Lys9	biotinidase	gene expression

TABLE 1-continued

Types of Histone Modifications, Sites of Modification, Proposed Functions and Enzymes Involved			
Histone	Site	Histone-modifying Enzymes	Proposed Function
	Lys18	biotinidase	gene expression
H4	Lys12	biotinidase	DNA damage response

In a preferred embodiment, the epigenetic modification is a modification to a histone. In another preferred embodiment, the histone modification is selected from the group consisting of acetylation, methylation, phosphorylation, ubiquitination, sumoylation and biotinylation. In yet another preferred embodiment, the histone is selected from the group consisting of H1, H2A, H2B, H3 and H4.

As shown in Table 1, histone modification can lead to both transcription activation and repression. In a preferred embodiment, histone modifications that lead to activation of TNF α transcription are analyzed. In another preferred embodiment, histone modifications that lead to repression of TNF α gene expression are analyzed.

Histone acetylation is a reversible modification of specific residues in histone "tails" and is controlled by histone acetyltransferases (HATs) and HDACs that typically act as transcriptional co-activators and co-repressors, respectively. In a preferred embodiment, the histone modification is acetylation. In another preferred embodiment, acetylation of histone H4 is analyzed. In yet another preferred embodiment, the presence of a HAT is analyzed. In still another preferred embodiment, the presence of a HDAC is analyzed.

Recent findings suggest that lysine and arginine-specific methylation of histones may cooperate with other types of post-translational histone modification to regulate chromatin structure and gene transcription. Reviewed by Stallcup, *Oncogene* 20:3014-3020 (2001). Proteins that methylate histones on arginine residues can collaborate with other coactivators to enhance the activity of specific transcriptional activators such as nuclear receptors. Lysine methylation of histones is associated with transcriptionally active nuclei, regulates other types of histone modifications, and is necessary for proper mitotic cell divisions. The fact that some transcription factors and proteins involved in RNA processing can also be methylated suggests that protein methylation may also contribute in other ways to regulation of transcription and post-transcriptional steps in gene regulation. cDNA clones for five genetically distinct but related mammalian arginine methyltransferases have been isolated: PRMT1, PRMT2/HRMT1L1, PRMT3, CARM1, and JBP1. Lin et al., *J. Biol. Chem.* 271:15034-15044 (1996); Scott et al., *Genomics* 48:330-340 (1998); Tang et al., *J. Biol. Chem.* 273:16935-16945 (1998); Chen et al., *Science* 284:2174-2177 (1999); Pollack et al., *J. Biol. Chem.* 274:31531-31542 (1999). Lysines may accept one, two, or three methyl groups on the terminal amine group of the lysine side chain. Histone H3 lysine methyltransferase activity has been observed in several proteins containing SET domains. Rea et al., *Nature* 406:593-599 (2000); O'Carroll et al., *Mol. Cell. Biol.* 20:9423-9433 (2000). The mammalian SET domain protein SUV39H1 methylates lysine 9 of histone H3, and the SET domain was important for this activity. The ability to methylate histones was observed in some but not all SET domain proteins.

In a preferred embodiment, the histone modification is methylation. In another preferred embodiment, the presence

of an arginine methyltransferase is analyzed. In yet another preferred embodiment, the presence of a lysine methyltransferase is analyzed.

Histone modifications provide the binding sites for a series of effector proteins that would affect chromatin function. Histone acetylation has been shown to facilitate the access of a number of transcription factors to the DNA. Lee, et al., *Cell* 72:73-84 (1993); Nightingale, et al., *EMBO J.* 17:2865-76 (1998); Vettese-Dadey, et al., *EMBO J.* 15:2508-18 (1996). Histone acetylation has also been shown to facilitate the passage of the RNA polymerase on chromatin. Marushige, *Proc. Natl. Acad. Sci. USA* 73:3937-41 (1976). Protein domains able to interact with chromatin and/or its modified components—like bromodomains, chromodomains or the SANT domains—can play a crucial role in the targeting process.

Histone modifications may promote transcription by providing binding sites for proteins involved in gene activation, such as those containing bromodomains. Shahbazian & Grunstein, *Annu. Rev. Biochem.* 76:75-100 (2007). The bromodomain is widely distributed among the different enzymes that acetylate, methylate or remodel chromatin. The chromatin remodeling complex SWI/SNF, which mobilizes nucleosomes and is important for transcriptional activation, appears to bind acetylated histones through a bromodomain; both the bromodomain of Snf2 and histone acetylation by SAGA or NuA4 are required for stabilization of SWI/SNF binding on nucleosome arrays. Hassan, et al. *Cell* 111:369-79 (2002). In mammalian cells, many transcription-promoting factors also contain bromodomains that bind specific acetylation sites. For example, the bromodomain in BRG1 (the catalytic subunit of SWI/SNF) has been shown to bind acetylated H4 K8, and the TBP-associated factor TAFII250 appears to bind H3 acetylated at K9 and K14. Agalioti, et al., *Cell* 111:381-92 (2002). Thus, in a preferred embodiment, the presence of a bromodomain protein is analyzed. In another preferred embodiment, the bromodomain protein is selected from the group consisting of BRM, BRG1, P300, PCAF, CBP, TAF250, GCN5, LOC330129 and MLL.

The chromodomain was first identified as a common domain between two distinct regulators of chromatin structure in *Drosophila*: HP1 and Polycomb. De la Cruz, et al., *BioEssays* 27:164-175 (2005). Later, chromodomains have been found in many other chromatin regulators: (i) remodeling factors involved in causing conformational changes by ATP-dependent movement of nucleosomes and (ii) histone acetyltransferases and methyltransferases. Jones, et al., *BioEssays* 22:124-137 (2000); Eissenberg, *Gene* 275:19-29 (2001). Recent work from different laboratories has shown that the HP1 chromodomain can recognize methylation of Lys 9 in histone H3, thus directing the binding of other proteins to control chromatin structure and gene expression. Apart from recognizing methyllysines, chromodomains can also serve to DNA and/or RNA recognition. The chromodomain has been found in HAT, HMT and ATP-dependent chromatinremodelling enzymes. De la Cruz, et al., *BioEssays* 27:164-175 (2005). Thus, in a preferred embodiment, the

presence of a chromodomain protein is analyzed. In another preferred embodiment, the chromodomain protein is selected from the group consisting of CHD-3, CHD-4, CHD-5, CDY-1, CDY-2, TIP60, MORF4L1, SUV39H1 and SUV39H2.

The SANT domain is present in some ATP-dependent remodelling enzymes complexes: yeast Swi3p, Rsc8p, BAF155/170 and Drosophila ISWI. De la Cruz, et al., *BioEssays* 27:164-175 (2005). The general role of the SANT domain is to stabilize, through direct binding, histone N-terminal tails in a conformation favoring their binding to the modifying enzymes, and the subsequent catalytic process. It has a central role in chromatin remodelling, being the unique histone-interaction module that couples histone binding to enzyme catalysis. It is present in the two enzyme classes responsible of chromatin modifications (enzymes that catalyse the histones covalent modifications and complexes using ATP hydrolysis). The preference of the SANT domain for unmodified histone tails suggests that histone deacetylation could increase its affinity for histone tails. Interaction with unacetylated histone tails could block the binding of HATs, thus maintaining the deacetylated state. Thus, in a preferred embodiment, the presence of a SANT domain protein is analyzed. In another preferred embodiment, the SANT domain protein is selected from the group consisting of SNF2L, SNF2H, EZH1 and EZH2.

The methylation of DNA is the covalent addition of a methyl group to the five-carbon (C⁵) position of cytosine bases in CpG dinucleotides. Methylation of cytosine is carried out by two types of DNA methyltransferases (DNMTs): de novo and maintenance methyltransferases. DNA methylation patterns are established during early development by de novo methyltransferases DNMT3A and DNMT3B. Patterns of DNA methylation are propagated with extreme fidelity by the maintenance methyltransferase DNMT1, which reproduces patterns of methylated and unmethylated CpG sites between cell generations. Thus, in a preferred embodiment, the epigenetic modification is DNA methylation.

Analyzing Epigenetic Modifications at the TNF α Locus

Histone modifications and DNA methylation at the TNF α locus may be analyzed by ChIP (chromatin immunoprecipitation) experiments. The sequence of the TNF α locus is provided as SEQ NO:1. To increase throughput, ChIP-on-chip experiments may be conducted to allow detection of histone modification or DNA methylation using microarrays or DNA chips with probes that cover the whole length of the TNF α locus or the entire promoter region.

In a preferred embodiment, the probes and primer pairs that are used for ChIP and ChIP-on-chip experiments are selected from the genomic sequences that cover the TNF α locus, including the promoter region, the exons, the introns, and the intergenic regions both upstream and downstream of the TNF α gene. DNA methylation may also be detected by bisulfite sequencing experiments or the HELP assay.

DNA methylation as well as histone acetylation and methylation have been shown to correlate with TNF α gene expression after PMA or LPS stimulation in K562 and THP-1 cells. See Sullivan, et al., *Mol. Cell. Biol.* 27:5147-5160 (2007). Alavi, et al. claims a method for determining potential TNF α expression in a cell population by determining if the TNF α promoter is methylated. WO 2008/137930. In THP-1 cells that are endotoxin-responsive, dissociation of heterochromatin-binding protein 1-alpha, demethylation of histone H3 lysine 9, increased phosphorylation of H3 serine 10, and recruitment of NF-B RelA/p65 to the TNF α promoter were shown to lead to increased TNF α mRNA. See Gazzar, et al., *J. Biol. Chem.* 282:26857-26864 (2007). Therefore, in a preferred embodiment of the invention the epigenetic modifica-

tion at the TNF α locus correlates with the level of TNF α gene expression after a Smac mimetic treatment. In another preferred embodiment of the invention, the histone acetylation at the TNF α locus correlates with the level of TNF α gene expression after a Smac mimetic treatment.

Also provided herein is a method to sensitize a cell population to apoptosis induced by Smac mimetics (as a single agent or in combination with other chemodrugs) by treating the cell population with an agent that changes the epigenetic modification pattern at the TNF α locus. In a preferred embodiment, the agent is an HDAC inhibitor. In another preferred embodiment, the agent is an inhibitor of DNA methyltransferase.

A relatively wide range of structures have been identified that are able to inhibit the activity of class 1, class 2 and class 4 HDACs. See Minucci & Pelicci, *Nat. Rev. Cancer* 6:38-51 (2006). They derive from both natural sources and from synthetic routes. The first category includes molecules such as FK-228, Apicidin, Trichostatin A (TSA), and sodium butyrate. The second category includes molecules such as SAHA, AN-9 (Pivanex), CHAPs, PXD-101, Sulfonamide hydroxamic acid, MS-275, Tubacin, sodium phenyl butrate, and sodium valporate, etc. With a few exceptions, they can all be divided into chemical classes including hydroxamic acid derivatives, carboxylates, benzamides, electrophilic ketones and cyclic peptides. In a preferred embodiment, the HDAC inhibitor is selected from the group consisting of MS-275, sodium butyrate, valproic acid, Trichostatin A, and suberoylanilide hydroxamic acid.

The human genome contains four DNA methyltransferase genes, DNMT1, DNMT2, DNMT3A, and DNMT3B, that encode proteins with distinct functional specificities. Stresemann, et al., *Cancer Res.* 66:2794-2800 (2006). The most widely used DNA methyltransferase inhibitor, 5-azacytidine (5-aza-CR), is a cytidine analogue that functions as a mechanism-dependent suicide inhibitor of DNA methyltransferases. To be effective, 5-aza-CR needs to be incorporated into DNA, which requires extensive modification of the compound through metabolic pathways. DNA methyltransferases recognize 5-azacytosine as natural substrate and initiate the methylation reaction. 5-aza-CR causes substantial cytotoxicity and is not a specific inhibitor of DNA methyltransferases. Due to its ribonucleoside structure, most of the compound becomes incorporated into RNA and thereby interferes with protein translation. This problem has been addressed by the development of a deoxyribonucleoside analogue, 5-aza-2V-deoxycytidine (5-aza-CdR), which becomes more directly incorporated into DNA and causes more efficient inhibition of DNA methyltransferases. Zebularine, procaine, EGCG, and RG108 are also known as DNA methyltransferase inhibitors. In a preferred embodiment, the DNA methyltransferase inhibitor is selected from the group consisting of 5-azacytidine, zebularine, procaine, EGCG, and RG108.

A further aspect of the invention is a method to induce apoptosis in a cell population by contacting the cell population with an apoptosis-inducing agent comprising a Smac mimetic and an agent that changes the epigenetic modification pattern at the TNF α locus. In a preferred embodiment, the agent is an HDAC inhibitor. In another preferred embodiment, the agent is a DNA methyltransferase inhibitor.

In preferred embodiments, the cells are in situ in an individual diagnosed as in need of an apoptosis promoting treatment, and the contacting step is effected by administering to the individual a pharmaceutical composition including a therapeutically effective amount of the Smac mimetic, wherein the individual may be subject to concurrent or antecedent radiation or chemotherapy for treatment of a neopro-

liferative pathology. In particular embodiments, the pathogenic cells are of a tumor, such as glioblastoma, astrocytoma, breast cancer, prostate cancer, lung cancer, pancreatic cancer, gastric cancer, colon cancer, ovarian cancer, renal cancer, hepatoma, melanoma, lymphoma, leukemia or sarcoma.

In additional embodiments, the target cells are pro-inflammatory cells or cells of tissue subject to pathogenic inflammation and/or autoimmunity. A wide variety of diseases involve such pathogenic inflammation, including rheumatoid arthritis, diabetes, asthma, lupus, myasthenia gravis, Graves disease, inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis and related conditions), pelvic inflammatory diseases, chronic obstructive pulmonary disease (COPD), chronic bronchitis, pneumoconiosis, pulmonary emphysema, interstitial lung fibrosis, allergic rhinitis (hay fever), inflammatory cardiovascular diseases (e.g., congestive heart failure and ischemia/reperfusion injuries), atherosclerosis (including coronary artery disease), stroke, neurodegenerative diseases, such as Alzheimer's disease, multiple sclerosis and amyotrophic lateral sclerosis (ALS), neuroinflammatory diseases, organ transplant rejection, autoimmune hematological disorders, psoriasis, scleroderma, chronic active hepatitis, primary biliary cirrhosis, glomerulonephritis, uveitis and keratoconjunctivitis.

The subject compositions encompass pharmaceutical compositions containing a therapeutically effective amount of an active, dimer-like Smac mimetic in dosage form, and a pharmaceutically acceptable excipient, such as a carrier or diluent. In some embodiments, such compositions also contain an additional therapeutic agent, such as an anti-neoproliferative chemotherapeutic agent, in addition to the Smac mimetic.

EXAMPLES

The following examples are provided to illustrate the invention and are not intended to represent or limit its scope. The Examples provide representative chemical syntheses, as well as assays for determining the bioactivity of Smac mimetics, e.g., as measured by IAP binding, procaspase-3 activation or promotion of apoptosis. These assays may also be used to screen for agents (e.g., antagonists) which potentiate such mimetic activity.

Tumor Necrosis Factor α Semiquantitative RT-PCR Protocol
This procedure describes the semi-quantitative measurement of human TNF α mRNA levels in human cell culture.

Materials:

For RNA isolation, RNAeasy miniprep kit from Qiagen or TRIzol Reagent from Invitrogen were used, along with 22-gauge needle, 1 cc syringe, and 70% EtOH.

For generation of cDNA, RNase free tubes, RNase free ddH₂O, Omniscript RT kit (Qiagen), and Oligo dT primer (IDT) were used.

For PCR Reaction, Thermocycler, Taq DNA polymerase (NEB), 10 mM dNTPS (NEB) were used.

Procedures:

Cell Treatment: cells were plated in 6 well dishes at 1.5×10^5 cells/ml in a volume of 2.5 ml, next day cells were treated +/-50 ul of 5 μ M Smac Mimetic, JP1010, (final concentration: 100 nM) for 4 hr.

RNA Isolation: cells were washed 2x with 2 ml PBS followed by isolation of RNA from 6 well dishes using Qiagen RNAeasy kit or TRIzol reagent (Invitrogen) according to manufacturer's protocol. RNA isolation with TRIzol was conducted by adding 1 ml of TRIzol reagent to each well and pipetting up and down several times before transferring lysate to 1.5 ml microfuge tube. The mixture was incubated at room

temperature for 5 min to allow for complete dissociation of nucleoprotein complexes followed by adding 0.2 ml of chloroform, vortexing for 15 sec and then incubating at room temperature for 2-3 min. Samples were centrifuged at $12,000 \times g$ for 15 min at 4° C. and the colorless upper aqueous phase was transferred to a new microfuge tube. RNA from the solution was precipitated by the addition of 0.5 ml isopropyl alcohol and incubation at room temperature for 10 min and centrifugation at $12,000 \times g$ for 10 min at 4° C. The supernatant was removed without disturbing the RNA pellet. The pellet was washed with the addition of 0.5 ml 75% ethanol followed by centrifugation at $7,500 \times g$ for 5 min at 4° C. The ethanol was removed and RNA pellet briefly air dried and then resuspended in 30 ul RNase free ddH₂O. RNA concentration was measured.

Generation of cDNA: 2 ug of total RNA was used in a 20 ul reverse transcription reaction with 2 ul 5 mM dNTPs, 2 ul $20 \times$ RT buffer, 2 ul Oligo dT primer, 1 ul diluted RNase Inhibitor (10 U/ul), 1 ul Omniscript RT, 2 ug RNA, up to 20 ul ddH₂O and incubated for 1 hr. at 37° C.

Semi-quantitative RT-PCR for TNF α and Actin were performed as follows: Control semi-quantitative Actin PCR were conducted in a 25 ul reaction mixture with the following cycle condition: 94° C. 2 min, 25 cycles of 94° C. 30 s, 52° C. 30 s, 72° C. 60 s, and 72° C. 7 min. The reaction mixture contained 1 ul cDNA template, 2.5 ul $10 \times$ Thermopolymerase buffer, 0.5 ul 10 mM dNTPS, 0.25 ul 100 uM Actin Forward primer, 0.25 ul 100 uM Actin Reverse primer, 0.5 ul Taq DNA polymerase, and 20 ul ddH₂O. TNF α semi quantitative PCR were conducted in a 25 ul reaction mixture with the following cycle conditions: 94° C.-2 min, 30-35 cycles (depending on cell line) of 94° C.-30 sec, 59° C.-30 sec, 72° C.-1 min, followed by 72° C. for 10 min. The reaction mixture contained 2 ul single strand cDNA template, 2.5 ul $10 \times$ Thermopolymerase buffer, 0.5 ul 10 mM dNTPs, 0.25 ul 100 uM TNF α forward primer, 0.25 ul 100 uM TNF α reverse primer, 0.5 ul Taq DNA polymerase, and 19 ul ddH₂O. After PCR was completed 5 ul of $6 \times$ DNA loading dye was added to each tube and 25 ul was loaded onto 1.5% Et-Br agarose gel and run for 30 min at 150V.

Chromatin Immunoprecipitation (ChIP) Protocol

Purpose: This procedure evaluates the localization of specific proteins or their modified forms on chromatin. For our purpose to specifically evaluate the levels of modified histones and cofactors bound to the Tumor Necrosis Factor (TNF α) gene promoter.

Materials:

ChIP Lysis buffer contained 1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH8.1, $1 \times$ protease inhibitor cocktail (Roche). ChIP Dilution buffer contained 1% Triton x-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, pH8.1, $1 \times$ protease inhibitor cocktail (Roche). Wash Buffer I contained 0.1% SDS, 1% Triton x-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl, pH8.1. Wash Buffer II contained wash buffer I with 500 mM NaCl. Wash Buffer III contained 0.25M LiCl, 1% NP-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH8.1. TE buffer contained 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA. ChIP Extraction buffer contained 1% SDS, 0.1M NaHCO₃. Antibodies used include anti-histone H3 (Abcam, ab1791), anti-hyperacetylated histone H4 (Millipore, 06-946), anti-dimethyl-histone H3k4 (Millipore, 07-030), anti-dimethyl histone H3K9 (Abcam, ab1220), anti-histone H4 (Millipore, 05-858), anti-acetylated H3 (Millipore, 06-599), anti-acetylated H4 (Millipore, 06-866).

Procedure:

Cells were plated on either 10 cm or 15 cm tissue culture dishes ($\sim 1 \times 10^6$). At 80-100% confluency cells were

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crosslinked by the addition of 37% formaldehyde (final concentration 1%) directly to the cell medium. Medium was removed and cells washed twice with ice cold PBS. Cells were scraped into a microfuge tube and pelleted by centrifugation at 2000 rpm for 5 min at 4° C. Pellet was resuspend in 200 ul ChIP Lysis buffer per 10 cm dish or 400 ul per 15 cm dish and let sit on ice for 10 min DNA was sheared to 200-1000 bp by sonicating the lysate on ice with a Branson Model 500 Sonic Dismembrator and microtip (power output: 37%, 4x10 sec pulses with 1 min on ice between pulses). Samples were centrifuged for 10 min at 13,000 g at 4° C. and supernatant transferred to a new 1.5 ml microfuge tube. 1/10 vol. of sonicated DNA was removed for INPUT and stored at -20° C. The remaining 180 ul of sonicated DNA was diluted into a total volume of 1.5 ml with ChIP Dilution Buffer. To reduce nonspecific background, diluted samples were pre-cleared with 50 ul of Salmon Sperm DNA/protein A Agarose-50% slurry (Upstate Biotechnology) for 30 min at 4° C. on Labquake tube rotator. Agarose beads were pelleted by brief centrifugation and supernatant transferred to a new microfuge tube containing the appropriate amount of immunoprecipitating antibody and incubate overnight at 4° C. with rotation. As a negative control, a no antibody or IgG immunoprecipitation was performed. 50 ul of Salmon Sperm DNA/protein A Agarose slurry was added to each tube and rotated for 1 hr at 4° C. to collect the antibody/histone complexes followed by centrifugation at 1000 rpm at 4° C. for 2 min Supernatant was aspirated off and beads were washed for 5 min on a rotating platform with 0.5 ml of each of the buffers listed in order as follows: Wash buffer I—Low Salt, one wash; Wash buffer II—High Salt, one wash; Wash buffer III—LiCl wash, one wash; TE buffer, two washes. Elute histone complex from antibody by adding 125 ul of ChIP extraction buffer and rotate at room temperature for 15 min Spin down beads and transfer supernatant to a new microfuge tube. Repeat elution step by adding 125 ul of ChIP extraction buffer and rotate 15 min. Combine the two elutions. Reverse crosslinking to isolate DNA by either of PCR purification kit or phenol/chloroform extraction. For DNA purification w/PCR kit add 5 ul RNase A (0.5 mg/ml) and heat at 65° C. overnight. DNA isolated with Qiagen PCR purification kit according to manufacturers protocol. (note: input samples from sonication step are removed from -20° C. and treated identically). For Phenol/Chloroform purification: sample volume (including inputs) are brought to a volume of 400 ul with ChIP extraction buffer and 5 ul of proteinase K (20 mg/ml) added. Heat overnight at 65° C. to reverse crosslinking and purify used standard phenol:chloroform extraction followed by ethanol precipitation with 10 ul of 5 mg/ml glycogen. Pellets were resuspended in final volume of 50 ul. PCR was performed using 2-5 ul of sample DNA in a 25 ul rxn as follows: 2.5 ul 10x Thermopolymerase buffer, 0.5 ul 10 mM dNTPs, 0.25 ul TNF α CHIP F primer, 0.25 ul TNF α CHIP R primer, 0.5 ml Taq DNA polymerase, and Up to 25 ul with ddH₂O. Thermocycler conditions: 95° C. for 2 min, followed by 34 cycles of 95° C. for 30 s, 51° C. for 30 s, 72° C. for 30 s. An additional 7 min incubation at 72° C. was performed. Gel Electrophoresis: After PCR was completed 5 ul of 6xDNA loading dye added to each tube and 25 ul was loaded onto 1.5% Et-Br agarose gel. Gel run for 30 min at 150V.

Note: the ChIP protocol was adapted from those described by Abcam, Upstate, and in *Biochem. Biophys. Res. Comm* 359:88-93 (2007).

Example 1

Acetyl-H4 Levels at the TNF α locus Correlate with Smac Mimetic Induced TNF-a Expression

Acetylated H4 levels were surveyed in cell lines that are either sensitive or resistant to Smac mimetic-induced TNF α

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expression. In the two cell lines that are inducible, A2058 and MB231, high levels of acetylated H4 were detected (FIG. 1). On the other hand, acetylated H4 was barely detectable in three cell lines that are non-inducible, MB231n1, G361, and H460 (FIG. 1).

Example 2

Higher Levels of Acetyl-H4 at the TNF α Locus Correlate with Smac Mimetic-Induced TNF-a Expression

An expanded panel of cell lines that are either sensitive or resistant to Smac mimetic-induced TNF α expression was surveyed for their acetylated H4 and acetylated H3 levels. Most sensitive cell lines showed a higher level of acetylated H4 than the resistant cell lines (FIG. 2). No significant difference in acetylated H3 levels was detected between the two types of cell lines (FIG. 2). Quantified Acetyl-H3 and Acetyl-H4 levels were normalized against total H3 levels.

Example 3

Smac Mimetics do not Affect Acetylated Histone H4 Levels at the TNF α Locus

The effect of Smac mimetic JP1010 on the level of acetylated H4 was tested in MDA-MB231 cells. The addition of 100 nM of JP1010 did not change the level of acetylated H4 either alone or in combination with 5 mM NaBu (FIG. 3). However, the HDAC inhibitor NaBu increased the level of acetylated H4 for 4-5 fold (FIG. 3).

Example 4

HDAC Inhibitor MS-275 Primes Colo205 Cells for Smac Mimetic Induced TNF-a Production

The effect on TNF α production by HDAC inhibitor MS-275 was examined in Colo205 cells. Both the levels of transcription, assayed by RT-PCR, and levels of protein secretion, assayed by measurement of secreted protein amount, were examined MS-275 increased production of TNF α mRNA, but not TNF α protein secretion (FIG. 4). It also increased the mRNA level of cIAP2 (FIG. 4A). When it was combined with the Smac mimetic JP1584, both the levels of TNF α transcription and protein secretion were increased (FIG. 4). However, JP 1584 alone did not increase the level of either mRNA or protein secretion of TNF α (FIG. 4).

Example 5

HDAC Inhibitor Sodium Butyrate (NaBu) Increases Smac Mimetic Induced TNF-a Production in H2073 Cells

The effect on TNF α production by HDAC inhibitor NaBu was examined in H2073 cells. Both the levels of transcription, assayed by RT-PCR, and levels of protein secretion, assayed by measurement of secreted protein amount, were examined. When NaBu was combined with the Smac mimetic JP1584, both the levels of TNF α transcription and protein secretion were increased (FIG. 5). However, JP 1584 alone did not increase the level of either mRNA or protein secretion of TNF α (FIG. 5).

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Example 6

Smac Mimetic does not Induce TNF- α Production in Resistant Cells

TNF expression in MDA-MB-231 and MDA-MB-231-N1 cells was analyzed by RT-PCR assay after treatment with 20 nM and 100 nM of Smac mimetic, JP1584. High level TNF α expression was detected in Smac mimetic-sensitive MDA-MB-231 cells even with 20 nM of JP1584 treatment, while no TNF α expression was detectable in the resistant MDA-MB-231-N1 cells (FIG. 6).

Example 7

Correlation between Induced TNF- α Expression and Gemcitabine/JP1584 Synergy

Twenty-six cells lines were surveyed for their JP1584-induced TNF α expression and sensitivity to Gemcitabine/JP1584-induced apoptosis. Eleven out of the twenty-six cell lines (42%) had JP1584-induced TNF α expression, while 15 (58%) did not (FIG. 7). Out of the 11 cell lines that had inducible TNF α expression, 9 (81%) were also sensitive to Gemcitabine/JP1584-induced apoptosis. Out of the 15 cell lines that had no inducible TNF α expression, only one (6.7%) was also sensitive to Gemcitabine/JP1584-induced apoptosis.

The above examples are included for illustrative purposes only and are not intended to limit the scope of the invention. Many variations to those described above are possible. Since modifications and variations to the examples described above will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

Citation of the above publications or documents is not intended as an admission that any of the foregoing is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

What is claimed is:

1. A method of inducing apoptosis in a cell population, which method comprises:
 - a) determining that the cell population is sensitive to Smac mimetic-induced apoptosis by detecting an epigenetic modification at the TNF α locus in the cell population correlated with sensitivity to Smac mimetic-induced apoptosis; and
 - b) contacting the cell population with a composition comprising an effective amount of a peptidyl Smac mimetic, whereby apoptosis is induced in the cell population.
2. The method according to claim 1, wherein the composition further comprises an effective amount of a chemotherapy drug.
3. The method according to claim 1, wherein the epigenetic modification is a histone modification.

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4. The method according to claim 1, wherein the epigenetic modification is a histone modification that correlates with Smac-induced TNF α gene expression.

5. The method according to claim 1, wherein the epigenetic modification is a histone modification that is acetylation.

6. The method according to claim 1, wherein the epigenetic modification is a histone modification that is acetylation of H4.

7. The method according to claim 1, wherein the epigenetic modification is a histone modification that is methylation.

8. The method according to claim 1, wherein the epigenetic modification is presence of a histone acetyltransferase or a histone deacetylase.

9. The method according to claim 1, wherein the epigenetic modification is presence of an arginine methyltransferase, a lysine methyltransferase, a bromodomain protein, a chromodomain protein, or a SANT domain protein.

10. The method according to claim 1, wherein the epigenetic modification is determined by chromatin immunoprecipitation, or by ChIP-on-chip.

11. The method according to claim 1, wherein the epigenetic modification is a histone methylation determined by bisulfite sequencing or the HELP assay.

12. The method according to claim 1, wherein the epigenetic modification correlates with the level of TNF α gene expression after a Smac mimetic treatment.

13. The method according to claim 1 wherein the contacting step further comprises contacting the cell population with an agent that changes an epigenetic modification at the TNF α locus.

14. The method according to claim 1, wherein the cell population is in situ in an individual diagnosed as in need of an apoptosis promoting treatment.

15. The method according to claim 13, wherein the agent is a histone deacetylase inhibitor.

16. The method according to claim 13, wherein the agent is a histone deacetylase inhibitor selected from the group consisting of MS-275, sodium butyrate, valproic acid, Trichostatin A, and suberoylanilide hydroxamic acid.

17. The method according to claim 13, wherein the agent is a DNA methyltransferase inhibitor.

18. The method according to claim 13, wherein the cell population is in situ in an individual diagnosed as in need of an apoptosis promoting treatment, and subjecting the individual to concurrent or antecedent radiation or chemotherapy.

19. The method according to claim 6, wherein the agent is a histone deacetylase inhibitor selected from the group consisting of MS-275, sodium butyrate, valproic acid, Trichostatin A, and suberoylanilide hydroxamic acid.

20. The method of claim 19 wherein the cell population is a tumor cell population isolated from a patient.

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