

CASPASES AND CANCER: MECHANISMS OF INACTIVATION AND NEW TREATMENT MODALITIES

Aleksei Philchenkov¹, Michael Zavelevich¹, Tadeusz J. Krocak², Marek Los^{2,3,}*

*¹R.E. Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology,
National Academy of Sciences of Ukraine, Kyiv 03022, Ukraine*

²Manitoba Institute of Cell Biology, Cancer Care Manitoba, Winnipeg, R3E 0V9, Canada

³Institute of Experimental Dermatology, University of Munster, Munster, D-48149, Germany

Elimination of superfluous or mutated somatic cells is provided by various mechanisms including apoptosis. Deregulation of apoptotic signaling pathways may contribute to oncogenesis. Aspartate specific cysteine proteases, termed caspases are the key effector molecules in apoptosis. The aim of this review is to summarize the various defects in caspase-dependent cell death machinery identified in the neoplastic cells. These include not only mutations, but also alterations of gene methylation, and altered mRNA stability. Among the molecules that we discuss are elements of the extrinsic death pathway like CD95 (APO-1/Fas), FADD, FLIPs, FLICE, other apical caspases, components of the intrinsic apoptotic pathway like Apaf-1, caspase-9, and modulators of apoptotic pathways like IAPs, Smac/DIABLO, OMI/HtrA2, and other apoptosis regulating proteins. We also discuss recent data on cancer-specific agents that target effector mechanisms of apoptosis. Particular emphasis is given to the prospects for combining cell suicide-activating approaches with classical cancer therapies.

Key Words: apoptosis, caspase, mutation, tumor cell, cancer patient, caspase activators, fusion proteins, cell-permeable peptides, anticancer drugs, gene therapy, siRNA, preclinical study.

Apoptosis is an energy-dependent, tightly regulated and selective physiologic process that governs the removal of supernumerary or defective cells. It occurs under normal physiologic conditions, but it can also be triggered by diverse pathologies. In healthy tissues, the main role of apoptosis is to maintain the optimal number of cells in tissues and organs by removing the redundant, damaged, or functionally abnormal cells [48]. The most prominent morphologic features of apoptosis are membrane blebbing, cell shrinkage and chromatin condensation. Internucleosomal DNA fragmentation results in the occurrence of the so called "DNA-ladder" upon the extraction and electrophoresis of DNA from apoptotic cells. Apoptosis can be induced by diverse stimuli including some cell damaging agents and cancer therapy [66, 90, 92]. Apoptotic cell death does not induce immune response. In contrast, necrosis predominantly represents a passive form of cell death induced mainly by non-physiological agents. It is accompanied by autolysis of the cell, frequently initiated

by damage to the plasma membrane. Necrosis is often marked by cell swelling, formation of vacuoles, and eventually leading to cellular and nuclear lysis. The released cellular content subsequently stimulates an inflammatory response at the site [64].

Apoptosis plays an important role in oncogenesis [45]. The dysregulation of the intrinsic apoptotic program is common in cancer cells. The resulting impaired removal of mutated cells is important for tumor progression due to the following reasons: (i) increased probability of preserving and propagating mutations and other genetic abnormalities that may lead to large-scale genomic instability; (ii) abolition of cell cycle checkpoints resulting in uncontrolled transition through these checkpoints without the repair of occurring DNA lesions; (iii) escape of malignant cells from antitumor surveillance provided by the immune effector cells; (iv) ability to acquire resistance to chemotherapeutic drugs and irradiation.

The role of defects in apoptosis signaling and their contribution to the development and progression of cancer has been thoroughly summarized in a number of monographs and reviews [2, 3, 63, 90, 123]. Despite the variety of apoptosis-initiating events, programmed cell death signaling pathways finally converge on a common effector cellular disassembly machinery mediated by the family of cysteinyl endopeptidases known as the caspases. The recent progress in our understanding of the role of caspases in the execution of apoptosis has created significant interest for academic researchers and the pharmacological industry. Caspases and their regulators become potentially attractive targets for the development of new cancer therapies. This review article attempts to summarize the recent strategies developed that explore our knowledge of apoptotic processes, targeting cancer cells. The

Received: January 17, 2004.

*Correspondence: Fax: +1 (204) 787-2190
E-mail: losmj@cc.umanitoba.ca

Abbreviations used: Apaf-1 — apoptosis protease-activating factor-1; ASC — apoptosis-associated speck-like protein containing CARD; CARD — caspase recruitment domain; CARDIAK — CARD-containing ICE-associated kinase; c-FLIP — cellular FLICE-inhibitory protein; c-IAP — cellular inhibitor of apoptosis; DD — death domain; DED — death effector domain; FADD — Fas-associated death domain protein; FLICE — FADD-like ICE (caspase-8); HtrA2 — high-temperature requirement factor A2; ICE — interleukin-1 β -converting enzyme (caspase-1); NAIP — neuronal apoptosis inhibitory protein; PACAP — proapoptotic caspase adaptor protein; RAIDD — RIP associated ICH-1/CED-3 homologous protein with a death domain; RIP — receptor interacting protein; Smac — second mitochondrial activator of caspases; XIAP — X chromosome-linked inhibitor of apoptosis.

new cancer treatment approaches are discussed in the context of existing, traditional cancer therapies.

Molecular anatomy of caspase-dependent apoptotic program. At the molecular level, the apoptotic cell death machinery forms a complex cascade of ordered events, which are controlled by the regulated expression of apoptosis-associated genes and proteins, including proteases, protein kinases, phosphatases, and endonucleases. It is the concerted action of these components that finally results in cell dismantling and in the formation of apoptotic bodies. The key components of this self-destructing machinery are the caspases. The importance of the caspase family of proteases has been effectively revealed by inhibitor studies. The irreversible pan-caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp fluoromethyl ketone (zVAD-fmk), and to a lesser degree subfamily-specific inhibitors, reduced both morphological and biochemical signs of apoptosis induced by death ligands, drugs, γ -radiation and a number of other stimuli [33, 66, 116, 117]. Nevertheless, the inhibition of caspases does not prevent cell death mediated by caspase-independent mechanisms. Caspases (cysteiny l aspartate-specific proteases) represent the family of cysteiny l endopeptidases, which cleave their substrates at specific aspartic acid residues. Twelve mammalian caspases presently known are numbered in the chronological order of their identification.

Caspases are synthesized as single-chain inactive zymogens consisting of four domains: a NH₂-terminal prodomain of variable length, a large subunit with molecular weight of about 20 kDa, a small subunit (~10 kDa), and a linker region connecting these catalytic subunits. The linker region is missing in some family members. Proteolytic cleavage of the caspase precursors results in the separation of large and small subunits with the production of a heterotetrameric complex (the active

enzyme) consisting of two large and two small subunits [100, 135]. Caspases differ in the length and in the amino acid sequence of their NH₂-terminal prodomain which is either short (20–30 amino acid residues) or long (Table 1). The long prodomain (more than 90 amino acid residues) contains one of two modular regions essential for interaction with adaptor proteins. These modules contain death effector domains (DED) and caspase recruitment domains (CARD). Two DEDs are found in both procaspase-8 and -10, while CARD is present in procaspase-1, -2, -4, -5, -9, -11, and -12. Hydrophobic protein interactions are mainly achieved via DED-DED contacts, whereas electrostatic interactions occur through CARD-CARD contacts.

Based on their proapoptotic functions, the caspases have been divided into two groups: initiators and effectors. First-group initiator (or apical) caspases (caspases-2, -8, -9, -10, and, probably, -11) activate the second-group of caspases (caspases-3, -6, and -7). The effector (or downstream) caspases are able to directly degrade multiple substrates including the structural and regulatory proteins in the cell nucleus, cytoplasm, and cytoskeleton. The proteolytic cleavage of cellular targets by effector caspases leads to the deregulation of vital cell processes and ultimately to cell death. In some cases, initiator caspases can also function as effector caspases. This activity helps to amplify a suicide signal in the cell whose death pathways have only been weakly initiated. Furthermore, the activation of effector caspases can not only be caused by initiator caspases, but also by other, non-caspase proteases, including cathepsins, calpains, and granzymes [44]. Caspase-1 and caspase-4, -5 have similar structures and are predominantly involved in the maturation of proinflammatory cytokines. Also, their role in the initiation and execution of apoptotic cell death cannot be excluded. So, for instance, IL-1 β maturation may

Table 1. Structural and functional characteristics of cysteine endopeptidases of the caspase family^a

Enzyme	Size of enzyme precursor (kDa)	Prodomain type	Active subunits (kDa)	Activation adaptor	Recognized substrate sequence
<i>Apoptosis-initiating caspases</i>					
Caspase-2	51	Long, with CARD region	19/12	RAIDD, PACAP, DEFCAP	DEHD ^b
Caspase-8	55	Long, with two DED-regions	18/11	FADD, DEDAF, ASC	LETD
Caspase-9	45	Long, with CARD region	17/10	Apaf-1, Nod-1, PACAP	LEHD
Caspase-10	55	Long, with two DED-regions	17/12	FADD, DEDAF	Unknown
Caspase-12	50	Long, with CARD region	20/10	TRAF-2	Unknown
<i>Effector caspases</i>					
Caspase-3	32	Short	17/12	NA ^c	DEVD
Caspase-6	34	Short	18/11	NA	VEHD
Caspase-7	35	Short	20/12	NA	DEVD
<i>Caspases involved in inflammation</i>					
Caspase-1	45	Long, with CARD region	20/10	CARDIAK, ASC, CARD-8, Ipaf, Nod-1	WEHD
Caspase-4	43	Long, with CARD region	20/10	Unknown	(W/L)EHD ^d
Caspase-5	48	Long, with CARD region	20/10	Unknown	(W/L)EHD
Caspase-11 ^e	42	Long, with CARD region	20/10	Unknown	(I/L/V/P)EHD
<i>Other mammalian caspases</i>					
Caspase-14	30	Short	20/10	NA	Unknown
<i>Invertebrate caspases^f</i>					
Ced-3	56	Long, with CARD region	17/14	Ced-4	DEVD
Dcp-1 ^g	36	Short	22/13	NA	DEVD
Dronc ^g	50	Long, with CARD region	20/14	DARK ^h	VQVAD

^a Adapted from Ref. 135 with modifications.

^b The sequence of amino acid residues is presented in P₄-P₁ direction; the proteolysis occurs after the aspartic acid residue in the P₁ position.

^c NA — not applicable.

^d In parentheses each amino acid possibility is listed in order of their preferential location.

^e Detected in murine cells.

^f Only several caspases are given as an example.

^g The *Drosophila* caspases.

^h A *Drosophila* homologue of Apaf-1.

be observed upon apoptosis induction by some stimuli [66]. Caspase-1 and -11 promote the activation of effector caspase-3 and -7, and to a significantly lesser extent caspase-6. Because caspase-11 is an upstream activator of caspase-1 and -3 [46], it may be assigned to initiator caspases.

The caspase proteolytic signaling cascades are interconnected and due to overlapping substrate specificity they are also partially redundant (see below). As a result, the apoptotic signal is greatly amplified, which is an event frequently observed if cellular or viral caspase inhibitors are not in place. Caspase-9 is necessary for the cytochrome *c*-dependent activation of caspase-2, -3, -6, -7, -8, and -10; caspase-3 is required for activating caspase-2, -6, -8, and -10 and subsequently for the cytochrome *c*-dependent activation of caspase-9 [112]; caspase-8 is able to activate *in vitro* seven zymogens of other caspases (procaspase-1, -2, -3, -6, -7, -9, and -11) [128]. In the final stage of caspase cascade, caspase-6 catalyzes the activation of caspase-8 and -10, and caspase-2, -7, -8 and -10 may directly cleave protein substrates [112]. Although caspase-2 is unable to initiate the processing of procaspases on its own, it stimulates the efflux of cytochrome *c* (and other proapoptotic mediators) from mitochondria by degrading Bid protein that promotes the activation of caspase-9 [32].

Perhaps the best defined caspase triggering cascade is the receptor mediated pathway. It is initiated by the binding of death ligands (belonging to the tumor necrosis factor [TNF]/nerve growth factor [NGF] superfamily) to the respective death receptor. To date, at least eight human members of the death receptor family have been identified: TNF-R1, Fas (Apo-1, CD95), DR-3 (Apo-3, WSL-1, TRAMP), DR-4 (TRAIL-R1), DR-5 (TRAIL-R2), DR-6, EDA-R (ectodermal dysplasia receptor), and NGF-R [25]. All death-inducing receptors contain a so called "death domain" (DD) in their cytoplasmic tail, which is a conserved stretch of about 80 amino acids. This structure is critical for engaging downstream molecules of the apoptotic cascade. The best characterized death-receptor signaling pathway is triggered after interaction of Fas(CD95/APO-1) with its ligand FasL. Ligation of Fas receptors leads to the formation of a multiprotein complex, DISC (Death Inducing Signaling Complex), that is essential for the initiation of apoptotic cascade [52]. Employing co-immunoprecipitation, two-dimensional electrophoresis and subsequent protein sequencing, Kischkel and his colleagues [52] have identified the critical components of DISC. Besides the trimer of death receptors that provide the framework for the recruitment of other proteins, DISC comprises Fas-associated proteins with DD (FADD) and with FADD-like IL-1 β -converting enzyme (FLICE, caspase-8).

Alternatively, caspase cascade may be initiated in a receptor-independent manner by a variety of stimuli, including chemotherapeutic agents. Proapoptotic signals can originate in various cellular organelles including the nucleus, mitochondria, the endoplasmic reticulum (ER),

lysosomes and the Golgi complex [24]. In the majority of these organelles, excluding perhaps the mitochondria, triggering mechanisms and underlying molecular networks are not known in details, however some facts are established. The nuclear protein p53 is a central link in the cellular mechanisms activated upon DNA injury promoting apoptosis through transcriptional activation/repression of various apoptosis-associated genes (Table 2). Moreover, some pathways that rely on p53-induced initiation of apoptosis and that do not depend on p53 transcriptional activity have been found [10].

Many apoptotic stimuli that induce metabolic stress in cell organelles will eventually converge on the mitochondria/apoptosome death pathway. Various inducers of apoptosis can directly or indirectly influence the permeability of the outer mitochondrial membrane and ultimately lead to the release of cytochrome *c*. The cytoplasmic efflux of cytochrome *c* is the key event in the activation of the mitochondria/apoptosome-dependent (intrinsic) death pathway. Bcl-2 family proteins are the major regulators of this pathway [79]. Their expression level and activation stage can strongly influence the release of a number of apoptogenic molecules like cytochrome *c*, procaspase-2, -3, -9, the apoptosis inducing factor (AIF), endonuclease G, Smac (second mitochondria derived activator of caspase)/DIABLO (direct IAP binding protein with low pl), Omi/HtrA2 (high temperature requirement A2), and many others from the mitochondrial intermembrane space. Among these events the activation of procaspase-9 (initiator of the intrinsic/apoptosome pathway) is perhaps the most important consequence. The activation is facilitated by Apaf-1 and cytochrome *c*, which in the presence of dATP or ATP form a multicomponent apoptosome complex with a molecular weight of approximately 700–1400 kDa [11]. Once activated, caspase-9 directly processes the downstream caspases-3 and -7 [100].

As mentioned above, the proteolytic processing and activation of initiator procaspases occurs within large multiprotein complexes like DISC and apoptosome. The involvement of adaptor molecules helps procaspases to align with proper spacing within these protein complexes [130]. Several adaptor proteins (RAIDD, FADD, Apaf-1, CARDIAK, DEFCAP, PACAP, DEDAF, Nod1, Ipad, ASC, CARD-8) have been found in vertebrates and one (Ced-4) in nematodes (see Table 1). Apoptosome formation may not be the sole mechanism of apical caspase activation. It appears that oligomerization represents a general mechanism for activation of all procaspases with long prodomains. Recently, Chang et al. [13] have shown that the aggregation of multiple procaspase-9 molecules can induce their activation without apoptosome.

The semi-hierarchical organization of caspase proteolytic cascades resembling for example the blood-coagulation system guarantees the rapid execution of apoptosis even if some family members are lacking. This has been elegantly demonstrated by a series of experiments involving immunodepletion of single caspases from cell extracts or by *in vitro* caspase assays as well

Table 2. Properties of some endogenous activators and inhibitors of caspases

Protein	Size (kDa)	Localization of gene	Regulation of apoptosis	Biological function
FADD	23	11q13.3	S/I*	Recruits caspase-8 or -10 to the death receptor-associated signaling complex DISC; performs the activation of procaspase-8, and -10; involves in the control of cellular activation and proliferation
Apaf-1	130	12q23	S	Facilitates procaspase-9 aut activation by oligomerizing its precursor molecules
Cytochrome c	12	7p15.2	S	Co-factor for Apaf-1; component of the mitochondrial respiratory chain
Smac/DIABLO	27.1	12q24.1–q24.31	S	Promotes cell death by preventing IAPs from binding to and inhibiting caspases
Omi/HtrA2	48.8	2p13.3	S	Augments caspase-dependent apoptosis by blocking IAPs and may induce caspase-independent cell death via its serine protease activity
ASC/TMS1	22	16p11.2–12	S/I	Activates procaspase-1; modulates NF-kappaB activation pathways
Bcl-2	26	18q21	I	Regulates the mitochondrial membrane permeability; blocks the release of apoptogenic proteins from mitochondrial intermembrane space; inhibits caspase activity by binding to Apaf-1
Bcl-x _L	26	20pter-p12.1	I	Prevents the release of the mitochondrial apoptogenic proteins into cytosol by blocking VDAC; forms heterodimers with Bak and Bcl-2; inhibits the association of Apaf-1 with caspase-9
Bax	21	19q13.3–q13.4	S	Binds to and antagonizes the Bcl-2 protein; promotes the release of apoptogenic proteins from mitochondrial intermembrane space resulting in caspase activation
Bad	18.4	11q12.3	S	Forms heterodimers with the antiapoptotic proteins (Bcl-2, Bcl-x _L , Bcl-w) and promotes the release of apoptogenic proteins from mitochondrial intermembrane space resulting in caspase activation
Bid	22	22q11.21	S	Forms heterodimers either with the proapoptotic protein Bax or the antiapoptotic protein Bcl-2; counters the protective effect of Bcl-2; promotes the release of apoptogenic proteins from mitochondrial intermembrane space resulting in caspase activation
p53	53	17p13.1	S	Induces apoptosis either by stimulation of <i>Fas</i> , <i>DR5</i> , <i>Apaf-1</i> , <i>procaspase-6</i> , <i>Bax</i> , <i>Bid</i> , <i>Noxa</i> , and <i>Puma</i> expression, or by repression of <i>Bcl-2</i> and <i>PTEN</i> ; directly induces permeabilization of the outer mitochondrial membrane by forming complexes with Bcl-x _L and Bcl-2 proteins; participates in cell cycle regulation
ciAP-1/MIHB/BIRC2	70	11q22–q23	I	Inhibits activity of caspase-3, and -7; interacts with Smac/DIABLO and inhibits its antiapoptotic activity; binds to TRAF-1 and TRAF-2
ciAP-2/MIHC/BIRC3	68	11q22–q23	I	Inhibits activity of caspase-3, and -7; interacts with Smac/DIABLO and inhibits its antiapoptotic activity; binds to TRAF-1 and TRAF-2
XIAP/MIHA/BIRC4	57	Xq25	I	Interacts with and inhibits caspase-3, -7 and -9; has ubiquitin ligase activity which promotes the degradation of caspase-3; interacts with Smac/DIABLO and inhibits its antiapoptotic activity; participates in the BMP/TGF-β signaling pathway
Survivin/BIRC5	16.5	17q25	I	Interferes with the activation of caspase-3, and -7; interacts with Smac/DIABLO; chromosomal passenger protein that is required for cell division
Livein/ML-IAP/BIRC7	31	20q13.3	I	Inhibits caspase-3 and proteolytic activation of procaspase-9; interacts with caspase-3, -7, and -9
c-FLIP	55	2q33–q34	S/I	Competes with procaspase-8 for binding to FADD and thereby inhibits procaspase-8 activation; controls the T-cell activation
ARC	30	16q21–q23	S/I	Interacts with caspase-2, and -8; inhibits caspase-8; prevents cytochrome c release; preserves mitochondrial function

* Abbreviations: BMP – bone morphogenetic protein; I – inhibition; S – stimulation; TGF – transforming growth factor.

as in murine caspase knock-out models [100, 112, 128]. Furthermore, absence of a single caspase in the system may trigger compensatory overexpression of other caspase family members [145]. For instance, neocarzinostatin-induced apoptosis in MCF-7 human breast cancer cells lacking active caspase-3 occurs via sequential activation of caspase-9, -7, and -6 [59]. Cisplatin-mediated apoptosis in testicular cancer cells with blocked caspase-9 is mediated via caspase-2 and caspase-3 dependent pathways [77].

Mechanisms that govern the induction of apoptosis in organelles like ER, Golgi apparatus, or lysosomes are much less clear. Upon increasing the intracellular Ca²⁺ content or inducing of ER stress (changes in Ca²⁺ metabolism and accumulation of the unfolded proteins in the ER), calpain or caspase-7 translocate to the ER surface where they process the precursor of caspase-12 with further activation of caspase-9 and caspase-3 (reviewed in [92]). Some Golgi-resident proteins are cleaved during apoptosis and facilitate the disassembly of the Golgi apparatus. The identification of goldgin-160 as the unique substrate for caspase-2 suggests that caspase-2 may also induce apoptosis independently of the mitochondria/apoptosome pathway [67]. Upon apoptosis induction, lysosomal enzymes, in particular proteases of the cathepsin family may enter the cytosol, facilitating cytochrome c release from the mitochondria [94]. Experiments with microinjections of cathepsin D into

the cytoplasm of human fibroblasts demonstrated the importance of this protease for the initiation of mitochondrial cytochrome c redistribution [95].

Individual caspases contribute to cell death machinery in cell-type and in a signaling cascade-specific manner. About 300 pro- and antiapoptotic protein substrates of caspases have been identified (see [19] for partial list). The substrate specificity of the above-listed caspases appears to be partially overlapping. The destruction of the nuclear matrix proteins results in the disruption of the structural organization of the nucleus and the condensation of chromatin, whereas the degradation of the cytoskeleton proteins (actin and actin binding proteins gelsolin and fodrin) contributes to the blebbing of the plasma membrane [100]. Lamins, NuMa, and Acinus, other nuclear substrates of caspases, are among the proteins responsible for maintenance of the nuclear integrity. The condensation and fragmentation of the nucleus observed during Fas-mediated apoptosis is usually preceded by the irreversible cleavage of the nuclear protein NuMa by caspase-6 [36]. Unlike lamins and NuMa, which are substrates of several caspases, Acinus is cleaved only by caspase-3 [101].

Among other caspase substrates are poly(ADP-ribose)polymerase-1 (PARP-1) and the DNA-dependent protein kinase (DNA-PK) that play an essential role in the repair of DNA lesions [96]. Caspase cleavage of PARP-1 or DNA-PK disrupts their ability to ac-

tivate DNA repair processes. Apoptotic internucleosomal DNA degradation is also regulated by caspase(s). The caspase-activated DNase (CAD) responsible for the internucleosomal DNA fragmentation is usually complexed in the cytoplasm with its inhibitor ICAD [23]. The degradation of ICAD that is processed mainly by caspase-3 liberates CAD, resulting in its nuclear transfer and subsequent DNA degradation.

Caspases also cleave and activate some protein kinases (e.g. MEKK-1, PKC- σ , or PAK2), whose activation contributes to the late events in apoptosis. Conversely, caspases inactivate a number of protein kinases, including Akt-1 and Raf-1, which are crucial for cell division and survival [132]. Finally, several suicide cell death antagonists are inactivated by caspases. For example, caspases cleave the antiapoptotic proteins Bcl-2 and Bcl-x_L as well as XIAP [17, 21, 51].

The antiapoptotic effect of endogenous caspase inhibitors is thought to be associated with their ability to inhibit either the activation of procaspases or the proteolytic effect of the active caspases. The first caspase inhibitors were detected among viral proteins responsible for survival of virus-infected cells [12]. The homologues of most of these virus-coding caspase inhibitors were later found in mammalian cells as the normal components of cell death machinery. So far, eight proteins similar to baculoviral IAPs (inhibitors of apoptosis) have been found in mammals [12, 93]. All the IAP family proteins share a specific BIR (baculoviral IAP repeat) region of about 70 amino acid residues. Human XIAP, c-IAP-1, c-IAP-2, and NAIP have three such motifs. At least one of the BIR regions is required to provide the antiapoptotic effect of these proteins. At the C-terminus of the IAP molecule a zinc RING-finger domain is found, which was not obligatory for inhibition of the apoptotic signal in the majority of cell types. Thus, c-IAP-1, c-IAP-2, and XIAP proteins retain their antiapoptotic function in the absence of the RING domain [97, 118]. The structure of c-IAP-1 and c-IAP-2 proteins is also characterized by the presence of the CARD domain located between the BIR and RING regions. The significance of this domain for IAP functions is not clear, although it is known to be required for c-IAP-1 binding to the CARD containing kinase CARDIAK/RIP2 involved in the activation of caspase-1 [70].

Interestingly, the XIAP protein that is the most efficient inhibitor among the family members can bind only to active forms of caspase-3 and -7, but not to their precursors. Moreover, the binding to the effector caspase-3 and -7 is due to the BIR2 domain of XIAP, whereas the BIR3 domain is essential for inhibition of the initiator caspase-9 [30]. The family of IAP proteins also includes a survivin, which contains only one BIR sequence. Survivin that may be preferentially expressed in tumor cells, binds to caspase-3 and -7 similarly to other proteins of the IAP family and inhibits the development of apoptosis induced by various stimuli [1]. The location of survivin in microtubules seems to promote its anticaspase activity during the G₂/M phase of the cell cycle.

Similarly to survivin, two recently found proteins of the IAP family, ILP-2 and livin (also called ML-IAP [melanoma-IAP] and KIAP [kidney-IAP]) contain only one BIR sequence [47, 93]. However, unlike survivin, both livin and ILP 2 are specified by the presence of the RING domain. Livin can inhibit the apoptosis mediated by death receptors and can also initiate it by overexpressing FADD, Bax, RIP, and RIP3 proteins [47]. Although ILP-2 fails to inhibit the Fas- or TNF-dependent apoptosis, it displays a pronounced antiapoptotic effect by counteracting cell death induced by overexpression of the Bax protein or by coexpression of caspase-9 with its adaptor Apaf-1 [93]. Both ILP-2 and livin can inhibit the initiator caspase-9. Moreover, similarly to other IAP proteins, livin can bind to the activated forms of caspase-3 and -7 [47].

The antiapoptotic effect of the IAP family proteins can be cancelled by specific inhibitors. After the initiation of apoptosis, Smac/DIABLO and Omi/HtrA2 proteins are released from mitochondria to the cytosol along with cytochrome *c*. Their N-terminal sequences contain a conserved AVPS motif which can bind IAP [69, 137]. The binding of Smac/DIABLO or Omi/HtrA2 to the XIAP protein countermands the caspase-inhibitory effect of the latter by promoting the activation of caspases. The overexpression of Smac/DIABLO or Omi/HtrA2 in the cells increases their sensitivity to induction of apoptosis by ultraviolet radiation [69, 129]. These findings confirm the capacity of these mitochondrial proteins to function as endogenous activators of apoptosis. The three-level regulatory mechanisms (caspase activators – caspase inhibitors – inhibitors of caspase inhibitors) that target caspase activity in a precisely regulated spatial and temporal manner, indicate the importance of these molecules in cell physiology. The limited activation of some caspase family members disengaged in time and restricted to certain cell compartments may be important for accomplishing cell functions other than apoptosis. This becomes crucial for various cells of the immune system since caspase activity is necessary for maturation of pro-IL-1 β , pro-IL-16, pro-IL-18 and pro-EMAP II (Endothelial Monocyte-Activating Polypeptide II) [5, 100].

In addition to classical inhibitors, most cells predominantly in the immune system express caspase-8 decoys called FLIPs (FLICE inhibitory proteins) [41]. FLIPs exist in two forms, the short one (FLIP_S) and the long one (FLIP_L). FLIP_S contain two effector DD regions, whereas FLIP_L also has a caspase-like domain, but lacks proteolytic activity. The FLIP protein binds with high affinity to the DISC, thus preventing activation of caspase-8 (and possibly caspase-10) and transduction of the proapoptotic signal from death receptors [102]. Interestingly, under certain experimental conditions the overexpression of FLIP_L facilitates rather than inhibits the activation of caspase-8, probably by assisting the trimerization of procaspase-8 molecules in the DISC. Some herpes viruses and molluscum contagiosum virus can produce antiapoptotic viral proteins, vFLIPs, that promote the survival of the infected cells [89].

The recently discovered DED-containing molecule BAR (bifunctional apoptosis regulator) can compete with FADD for binding to procaspase-8 or -10, and can prevent their Fas-mediated activation [144]. Due to the presence of the transmembrane domain and SAM (sterile alpha motif)-region, the BAR protein interacts with the apoptotic proteins Bcl-2 and Bcl-x_l and thus prevents Bax-induced cell death. These features of BAR determine its unique ability to inhibit cell death in response to exogenous (death receptors) or endogenous (Bax-facilitated) stimuli [144]. Similarly to a multidomain BAR protein, another inhibitor of caspase activation called ARC (apoptosis repressor with CARD) [57] interacts with caspase-8 (but not with caspase-9, -3, or -1). Unlike BAR, the ARC protein contains the CARD region at its N-terminus. The expression of ARC is tissue-specific and occurs in skeletal and cardiac muscle [57]. This suggests the selectivity of the anti-apoptotic effects of ARC, especially in the case of cell death mediated by death receptors.

Functional defects of caspase activation in malignant cells. The recent data obtained in both *in vitro* and *in vivo* models confirm contribution of deregulated apoptotic pathways for cancer development and progression [34]. Inactivation of proapoptotic and/or activation of antiapoptotic components of cell death machinery have been found in a number of cancers (for overview see Table 3). The level of caspase-1, -2, -3, -6, -7, -8, -9, and -10 expression in cancer cell lines and/or neoplastic tissues was shown to be lower than in the control specimen or in morphologically normal peritumoral tissue samples [15, 20, 37, 54, 58, 62, 78, 84, 91, 110, 114, 121, 134]. It has been demonstrated that the restoration of caspase-3 expression in caspase-3-deficient cancer cells augments their sensitivity to undergo apoptosis in response to chemotherapeutic agents or to other apoptotic inducers [20].

The downregulation of apoptosis in malignant cells may also be caused by a reduced recruitment of the

initiator caspase-8 or -9 to the DISC and apoptosome protein complexes. This results in the impaired formation of death-inducing signaling complexes [4, 61]. Such a preexisting impairment seems to predispose to the development of malignancy in some lymphoma and ovarian cancer patients. Also, functional blocks in the extrinsic (death receptor) and/or the intrinsic (mitochondrial) apoptotic pathways might explain poor responses to chemotherapy in some cases [103].

Besides the impaired expression of caspases, the mutations of caspase genes contribute to the pathogenesis of both solid and hematologic malignancies. Soung and co-workers [115] have recently described somatic mutations of the *caspase-7* gene in colon carcinomas (2%), esophageal carcinomas (2%) and head/neck carcinomas (3%). Expression of tumor-derived caspase-7 mutants in 293 cells resulted in apoptosis suppression giving the evidence of the dominant/inactivating nature of these mutations.

An examination of 180 colorectal tumors using polymerase chain reaction, single-strand conformation polymorphism, and conventional DNA sequencing revealed frame-shift (1), nonsense (1), and missense (3) mutations of the *caspase-8* gene in 5.1% of invasive carcinomas. Such mutations were absent in all studied adenoma specimens [49]. The authors also found that in 60% of cases these mutations markedly decreased the activity of caspase-8. Three different mutation hot spots have been reported in the *caspase-8* gene. Firstly, mutation modifying the stop codon of *caspase-8*, thus extending the encoded sequence by Alu repeats were found in a head and neck cancer cell line BB49-SCCHN [68]. Secondly, in a neuroblastoma cell line the missense mutation (alanine → valine) at codon 96 was revealed [120]. Finally, caspase-8 mutant with deletion of leucine 62 has been recently identified in human vulvar squamous carcinoma A-431 cells [60]. These data indicate that *caspase-8* gene mutations may contribute to the pathogenesis of diverse carcinomas.

Table 3. Patterns of changes in expression of caspases and their endogenous modulators in human malignancies

Protein and/or gene	Pro- or anti-apoptotic activity	Type of change in malignant tissues	Cancer	References
Caspase-1	Proapoptotic	Decreased	Prostate cancer; colon cancer	[134]
Caspase-2	Proapoptotic	Decreased	Mantle cell lymphoma	[37]
Caspase-3	Proapoptotic	Decreased	Breast cancer; RCC*; prostate carcinoma; cervical carcinoma; basal cell ameloblastomas; relapse in childhood ALL	[15, 20, 54, 58, 91, 114]
Caspase-6	Proapoptotic	Decreased	Cervical squamous cell carcinoma	[15]
Caspase-7	Proapoptotic	Decreased	Colon cancer	[84]
Caspase-8	Proapoptotic	Decreased	Childhood neuroblastomas; RCC; SCLC; familial lymphoma patients	[4, 54, 110, 121]
Caspase-9	Proapoptotic	Decreased	Colon cancer	[84]
Caspase-10	Proapoptotic	Decreased	Cervical carcinoma; gastric carcinoma; RCC; SCLC; NSCLC	[54, 62, 78, 110]
Apa1	Proapoptotic	Decreased	Malignant melanoma; ovarian cancer	[83, 113, 136]
FADD	Proapoptotic	Decreased	Thyroid carcinoma; tongue carcinoma; mantle cell lymphoma	[37, 74, 124]
Smac	Proapoptotic	Decreased	Lung and prostate carcinomas; malignant schwannoma; rhabdomyosarcoma; malignant fibrous histiocytoma; leiomyosarcoma; angiosarcoma; liposarcoma; hepatocellular carcinoma	[81, 142]
ASC/TMS1	Proapoptotic	Decreased	Breast cancer; gastric cancer; melanoma; lung cancer	[31, 76, 131]
XIAP	Antiapoptotic	Increased	NSCLC; transitional cell carcinoma of the upper urinary tract; AML	[6, 75, 140]
Survivin	Antiapoptotic	Increased	Tumors of lung, breast, colon, stomach, esophagus, pancreas, liver, uterus, ovaries; neuroblastoma; pheochromocytoma; soft-tissue sarcoma; brain tumors; melanoma; Hodgkin's disease; non-Hodgkin's lymphoma; leukemias; MDSRA	[1]
c-FLIP	Antiapoptotic	Increased	Hepatocellular carcinoma; SCLC; pancreatic cancer; malignant melanoma; colon carcinoma; familial lymphoma patients; Hodgkin's disease	[4, 9, 22, 82, 99, 110, 122]

* Abbreviations: ALL — acute lymphoblastic leukemia; AML — acute myelogenous leukemia; MDSRA — myelodysplastic syndrome with refractory anemia; NSCLC — non-small cell lung cancer; RCC — renal cell carcinoma; SCLC — small cell lung cancer. The list of caspases and their endogenous modulators is not exhaustive.

Somatic mutations in the *caspase-5* gene coding region were identified by the combination of the polymerase chain reaction, single strand conformation polymorphism analysis and direct sequencing in two out of thirty lung cancer patients. In the same specimens no mutations were found in *hMSH3*, *hMSH6* or *Bax* genes [39]. The data suggest that in lung cancer cells, *caspase-5* might be a candidate for a tumor suppressor gene. Frame-shift mutations in the *caspase-5* gene have also been revealed in endometrial, colon, and gastric cancers (28%, 62%, and 44% respectively) exhibiting a microsatellite mutator phenotype [104].

Mutations of *caspase-10* gene have been found in gastric cancer tumor samples. The inactivating mutations were mainly localized in the coding regions of DED (codon 147) and the p17 large protease domain (codons 257 and 410) [85]. Interestingly, the same study has not revealed any mutations of *caspase-8* genes. The increased frequency of *caspase-10*, *Fas*, and *FADD* gene mutations in the metastatic lesions of non-small cell lung cancers as compared with that in the primary tumors suggests the possible role of proapoptotic gene inactivation in metastasizing [108]. Alternatively, mutations in these genes may indicate adaptation of metastasized cancer cells to the absence of growth and survival stimuli that were present in the primary tissue, but are no longer available in the new environment.

Mutations in caspase genes have also been identified in hematological malignancies. Six out of 12 leukemia and lymphoma cell lines having microsatellite instability showed frame-shift mutations in the *caspase-5* gene [119]. Mutations in the *caspase-5* coding region were found in 37.5% of samples in human T-cell lymphoblastic lymphoma studied. Together with the accompanying mutations in *TGF β -RII* it suggests their possible involvement in the pathogenesis of this malignancy [105]. Inactivating mutations of the *caspase-10* gene have been recently detected in non-Hodgkin's lymphomas [107]. Most of the mutations identified were in the p17 large caspase-10 subunits, while the remaining mutations were found in the coding regions of the pro-domain and the small subunits of caspase-10.

The changes in the methylation profile of the promoters are known to represent an important pathway for the repression of gene transcription in cancers. Until recently, there were no reports on promoter hypermethylation of caspase genes in solid tumors. However, some of the latest studies suggest that gene methylation is involved in the inactivation of caspase-8. The aberrant hypermethylation of 11 genes, including *caspase-8*, was shown in a series of 44 neuroblastic tumors [29]. Methylation-specific polymerase chain reaction after treatment of DNA with bisulphite was used to visualize methylation of CpG islands of tested genes. The authors found that at least one of eleven genes was hypermethylated in 95% (42 of 44) of cases. The frequency of altered methylation of the *caspase-8* gene was nearly 14%. In contrast, no hypermethylation was observed in four control normal tissue samples (brain and adrenal medulla) [29].

Inactivation of the *caspase-8* gene through DNA methylation has also been observed in some pediatric tumors, including rhabdomyosarcomas, medulloblastomas, retinoblastomas, and neuroblastomas [35]. Interestingly, methylation of the *caspase-8* gene was highly correlated with the methylation of the tumor suppressor *RASSF1A* gene. These data and resistance of caspase 8-null neuroblastoma cells to death receptor-mediated or doxorubicin-induced apoptosis [121] suggested that caspase-8 may be involved in the development and progression of some childhood CNS cancers.

A separate study reported the methylation of CpG islands of *Fas*, *TRAIL-R1*, and *caspase-8* genes in small cell lung cancer cell lines and the respective tumor samples, whereas these genes were not methylated in non-small cell lung cancer samples [38]. It should be noted that co-treatment of small cell lung cancer cells with the demethylating agent 5'-aza-2'-deoxycytidine and IFN- γ , restored *Fas*, *TRAIL-R1*, and *caspase-8* expression and increased sensitivity to *FasL*- and *TRAIL*-induced cell death. Moreover, the abnormal methylation profile of the *caspase-8* promoter was recently found in human hepatocellular carcinomas [143]. The downregulation of expression of other caspases in cancer cells have also been observed. For example, the posttranscriptional inactivation of caspase-10 has been reported in many pediatric tumor cell lines [35]. The above examples indicate that inactivation of caspase family members in cancer cells enables them to evade cell death pathways.

Caspase expression may also be regulated at the transcriptional level by alternative splicing. Truncated variants of caspase-2, -3, -8, or -9 generated in such a way may act as endogenous caspase inhibitors (for review see [88]). As a result, the overall activity of caspases in malignant tissues decreases and the activation threshold increases, thus leading to the prevention of apoptosis. This type of caspase activity downregulation has been detected in various human gastric cancer-derived cell lines where the truncated caspase-9 lacking its catalytic domain contributes to the resistance against apoptotic stimuli [42].

Yet another mechanism for downregulating caspase activity involving the increased expression of the endogenous inhibitors of caspases in cancer cells can be applied. The data summarized in Table 3 indicate examples of intracellular changes that lead to apoptosis resistance. Thus, in several cancer cell lines and primary tumor samples the level of antiapoptotic genes and/or proteins such as XIAP, Survivin, c-FLIP, as well as Bcl-2, Bcl-x_L, and Bcl-w increases, while the expression of proapoptotic caspase activators (*Apaf-1*, *FADD*, *Smac/DIABLO*, *ASC/TMS1*) decreases.

Similarly as it was indicated above with respect to caspase family members, several tumors inactivate other components of the apoptotic pathway by the abnormal methylation of the respective genes. For example, Soengas et al. [113] observed that the treatment of highly chemoresistant metastatic melanomas with the methylation inhibitor 5-aza-2'-deoxycytidine, restored phys-

iological levels of Apaf-1 and sensitivity towards chemotherapy. Methylation within the *Apaf-1* promoter region was also demonstrated in acute myelogenous leukemia, chronic myeloid leukemia and acute lymphoblastic leukemia [26]. Also, frequent hypermethylation of the proapoptotic *TMS1* gene that encodes for CARD-containing protein was observed in breast, gastric, and colorectal cancer cells [76]. Thus, besides the altered caspase expression, the malfunctions in their regulation by endogenous inhibitors or upstream activators at genomic, transcriptional, and posttranscriptional levels may contribute to the loss of caspase activity resulting in resistance to apoptosis, which appears to be of high importance for pathogenesis of both solid tumors and hematological malignancies.

Caspase-targeted modalities of cancer treatment. Despite the growing number of chemotherapeutic drugs on the market, drug targeting and selectivity has not been satisfactory so far and novel approaches that target cancer cells selectively need to be developed. Below, we discuss various novel strategies that force caspases and other components of the apoptotic machinery to achieve either selectivity, inducibility (fine control) of treatment, or reversal of resistance of cancer cells to existing therapy protocols.

Caspases or their endogenous regulators represent promising therapeutic targets since proteolytic auto-amplifying pathways once activated cannot be easily stopped, or reversed. Selective activation of caspases or at least lowering their activation threshold might help to combat malignancies. Diverse strategies designed to activate caspases and stimulate apoptosis in cancer cells are currently under experimental study (Table 4). One of such apoptosis-triggering approaches is based on fusion proteins that contain effector caspases. Jia and co-workers [43] have generated a chimeric protein, called immunocasp-3 that comprises a single-chain anti-erbB2/HER2 antibody and an active caspase-3 molecule. Upon transfection with the *immunocasp-3* gene, cells express and secrete the chimeric protein, which then binds to HER2-overexpressing tumor cells facilitating intracellular penetration of fusion protein. Subsequent cleavage of the constitutively active caspase-3 domain from the immunocasp-3 molecule and its release from internalized vesicles leads to apoptotic cell death in the tumor. Significant tumor regression in mouse xenografts of HER2-positive tumor cells was seen upon intravenous injection of Jurkat cells transduced with chimeric *immunocasp-3* gene expression vector as well as intramuscular or intratumoral injection of immunocasp-3 expression plasmid DNA. Other authors have shown that specific binding between intracellular antibody-caspase-3 fusion proteins and a respective multivalent antigen results in autoactivation of caspase-3 due to the close proximity of caspase-3 molecules. Such an autoactivation of caspase-3 triggers apoptosis and irreversibly kills CHO cells transfected with antibody-caspase-3 fusion protein-expressing plasmid [125]. Caspase-3 fused with antibodies directed towards extra- or intracellular tumor-specific proteins seems to

provide a compelling rationale for selective induction of apoptosis in cancer cells.

Pharmacological activation of caspases using small molecules might prove to be another effective approach to kill cancer cells, or at least to reverse the resistance to anticancer drugs. Caspase-3 is kept in an inactive stage by an intramolecular electrostatic interaction facilitated by a triplet of aspartic acid molecules, called the “safety-catch” [98]. Attempts have been made to design small pharmacologically active molecules capable of lowering activation threshold, or even activating the caspase on its own. Maxim Pharmaceutical, Inc. (<http://www.maxim.com>) has developed a pharmacologically-active caspase activator MX-2060. Comparable approach towards selective activation of caspase-3 by “small molecules” is followed by Merck Frosst (<http://www.merckfrosst.ca>). “Small molecule” caspase activators are peptides, which contain arginine-glycine-aspartate (RGD) motif. They exhibit marked proapoptotic properties and can directly induce auto-processing (auto-activation) of procaspase-3 [8]. RGD-containing synthetic peptides have also been shown to increase drug sensitivity of cancer cells due to triggering caspase-3 activation or lowering activation threshold [7]. Similarly, cell-permeable SmacN7(R)8 peptide, which disrupted XIAP binding with caspase-9, and cancels its inhibition, could reverse the resistance of non-small cell lung cancer H460 cells to chemotherapeutic drugs *in vitro* and *in vivo* [140].

Several gene therapy approaches have been aimed at replacing the defective caspases or their upstream activators in cancer cells by their normal counterparts. A variety of replication-incompetent adenoviral vectors carrying different caspase genes, including *caspase-3*, *-6*, *-8*, and *-9* have been generated and their antitumor activity have been assayed (see **1, 5, 7, 9, 11** in Table 4). Both *in vitro* and *in vivo* studies demonstrated antitumor activity of these vectors [71, 106, 126, 127]. The genes coding for effector caspases are preferably used in such constructs since apoptosis induced by the effector caspases is independent from the upstream initiating pathways. Komata et al. [55] demonstrated that an expression vector consisting of the constitutively active caspase-6 under human telomerase reverse transcriptase (hTERT) promoter triggers apoptosis in malignant glioma cells, but not in hTERT-negative normal cells. The tumor-specificity of this approach is driven by the expression of telomerase that is largely restricted to neoplastic cells.

The direct or indirect caspase activators (Apaf1, FADD, and Smac/DIABLO) have also attracted significant attention as potential targets for anticancer gene therapy (**11–14, 22, 23**). Transfer of the gene encoding Smac sensitized various cancer cells *in vitro* for drug-induced apoptosis [28, 72]. Similarly, *Apaf-1* gene transfer markedly enhances chemosensitivity of several transplanted tumors [109, 113]. Delivery of the wild-type genes of several caspase activators into cancer cells lacking functionally active counterparts restored their activity and induced apoptosis [50, 53]. This ap-

Table 4. Summary of *in vitro* and *in vivo* studies on antitumor efficacy of novel agents targeting caspases or modulators of caspase activity (2001–2003)

No	Target	Target-based agents	<i>In vitro</i> results	<i>In vivo</i> results	References
1	Caspase-1, caspase-3	Replication-deficient adenoviral vectors Ad-G/Casp1 or Ad-G/iCasp3 + CID*	Induction of apoptosis in human prostate cancer LNCaP and PC-3 cells	Inhibition of growth and decrease in volume of TRAMP-C2 tumors	[106]
2	Caspase-3	Eukaryotic expression vector pcDNA/Rev-Caspase-3	Decrease in growth and induction of apoptosis in gastric cancer SGC7901 cells	—	[27]
3		Vector expressing chimeric immunocasp-3 gene	Selective death of tumor cells overexpressing HER2	Tumor regression in a mouse HER2-positive xenografts	[43]
4		ScFv-caspase-3 fusion proteins	Selective killing of chinese hamster ovary CHO cells	—	[125]
5	Procaspase-3 and survivin	Adenoviral vectors Ad Caspase-3 + Ad Survivin T34A	Induction of apoptosis in ovarian carcinoma cell lines	Increase in survival of murine intraperitoneal ovarian carcinoma	[71]
6	Caspase-6	Expression vector hTERT/rev-caspase-6	Induction of apoptosis in hTERT-positive malignant glioma cells	Suppression of growth in subcutaneously established tumors in nude mice	[55]
7	Caspase-8	Adenoviral vector	Augmentation of X ray-induced apoptosis in colon cancer DLD-1 cells	—	[126]
8		Expression system hTERT-378/caspase-8	Induction of apoptosis in human malignant glioma cells	Inhibition of growth in subcutaneously established U373-MG tumors in mice	[56]
9		Adenoviral vector	Significant induction of apoptosis in colon cancer DLD-1 cells after combination treatment with 5-Fu	—	[127]
10	Caspase-9	Expression system caspase-9/FKBP12 + CID	—	Intraperitoneal injection of CID induced apoptosis of endothelial cells expressing iCaspase-9 and elimination of human microvessels engineered in immunodeficient mice	[80]
11	Caspase-9, APAF1	Adenoviral vectors Adv-Casp9 and Adv-APAF1	Augmentation of sensitivity of U373-MG glioma cells to etoposide-induced apoptosis	—	[109]
12	APAF1	Retroviral vector pBabe/puro/APaf-1	Enhancement of chemosensitivity in melanoma cell lines	—	[113]
13	FADD	hTERT/FADD construct	Induction of apoptosis in telomerase-positive tumor cells of wide range	Suppression of subcutaneous tumor growth in nude mice	[53]
14		Replication-deficient adenoviral vector	Increased cell death in A549 and NCI-H226 lung carcinoma cells	—	[50]
15	Survivin	Replication-deficient adenovirus pAd-T34A	Induction of apoptosis in cell lines of breast, cervical, prostate, lung, and colorectal cancer; enhancement of taxol-induced cell death	Suppression of <i>de novo</i> tumor formation, inhibition of the growth in established tumors and reduction of intraperitoneal tumor dissemination in breast cancer xenografts in immunodeficient mice	[73]
16		Antisense oligonucleotide	Induction of apoptosis in human mesothelioma H28 cells	—	[138]
17		Retroviral vector encoding a survivin-targeted ribozyme	Increase in apoptosis of human prostate cancer DU145 and PC-3 cells; increase in the susceptibility to cisplatin-induced apoptosis	Prevention of tumor formation in androgen-independent prostate cancer xenografts in athymic nude mice	[87]
18	XIAP	Antisense oligonucleotide G4 AS ODN	Induction of cell death in human NSCLC NIH-H460 cells; their sensitization to the cytotoxic effects of doxorubicin, taxol, vinorelbine, and etoposide	Inhibition of H460 solid tumor growth in a xenograft model; in combination with vinorelbine significant delay in tumor growth	[40]
19		Antisense oligonucleotide xiap AS PODN	Induction of apoptosis in multidrug-resistant bladder cancer T24 cells; escalation of doxorubicin-induced apoptosis	—	[6]
20	FLIP	siRNA	Sensitization of human tumor SV80 and KB cells for TRAIL-induced apoptosis	—	[111]
21		cFLIP antisense oligodeoxynucleotide	Sensitization of human hepatocellular carcinoma HLE cells to Fas-, TNF-R-, and TRAIL-R-mediated apoptosis	—	[81]
22	Smac	Recombinant adenovirus Ad CMV-Smac	Increase in apoptosis of ovarian carcinoma cells; sensitization of ovarian carcinoma cells to cisplatin and paclitaxel	—	[72]
23		pcDNA3.1 vector containing full-length Smac cDNA; pEGFPc1 vector encoding cytosolic Smac	Sensitization of various tumor cells for apoptosis induced by death-receptor ligation or cytotoxic drugs	—	[28]
24		Cell-permeable peptides	Sensitization of various tumor cells for apoptosis induced by death-receptor ligation or cytotoxic drugs	Enhancement of the antitumor activity of TRAIL in an intracranial malignant glioma xenografts; eradication of established tumors and survival of mice upon combined treatment with Smac peptides and TRAIL without detectable toxicity to normal brain tissue	[28]
25		Cell permeable peptide SmacN7(R)8	Selective reversion of the apoptosis resistance in human NSCLC NCI-H460 cells	Regression of tumor growth in combination with chemotherapy with little toxicity to the mice	[140]
26	Omi/HtrA2	siRNA	Desensitization of cells to TRAIL-induced apoptosis	—	[141]

* Abbreviations: CID — lipid-permeable chemical inducer of dimerization; hTERT — human telomerase reverse transcriptase; NSCLC — non-small cell lung cancer; siRNA — small interfering RNA.

proach appears to have a potential as a novel therapy for cancer when coupled with cancer selective targeting techniques.

Another promising strategy in gene therapy of cancer is based on the development of caspase constructs with inducible caspase-1, -3 or -9 molecules that are

activated “on demand” *in vivo* by addition of a cell-permeable chemical inducers of dimerization (so called dimerizers) specific for the given construct (1, 10). The approach has already been successfully tested in different experimental models. For example, the single intraperitoneal injection of dimerizer together with such constructs is sufficient for dramatic suppression of LNCaP (prostate cancer) tumor growth in nude mice, thus leading to a significantly increased survival of the tumor-engrafted animals [139]. In addition, controlled activation of an inducible *caspase-9* gene in neovascular endothelial cells in the tumor using the described above approach is being tested as a novel tumor-directed anti-angiogenic therapy [80].

Tools have been developed to modulate the expression of genes that may act as potential anticancer therapy targets. Blocking gene expression of caspase inhibitors using antisense oligonucleotides, catalytic ribozymes and antisense RNAs (see below) is potentially another powerful strategy for cancer therapeutics. RNA interference is a newly discovered and exciting technique that allows selective induction of degradation of cognate mRNA. For that purpose, a so called small interfering RNAs (a short double-stranded RNA oligonucleotides known as siRNAs) complementary to target messenger RNA molecules are applied. The antisense oligonucleotides downregulating genes of caspase inhibitors overexpressed in cancer, such as *survivin*, *XIAP*, and *FLIP* have been shown to directly facilitate apoptosis in several tumor models (16, 18, 19, 21). Williams et al. [133] have shown that siRNA-mediated disruption of survivin mRNA severely reduced colon tumor growth both *in vitro* and *in vivo* xenograft models. A similar experiment using siRNA technology provides direct evidence that the intracellular interference with FLIP (20), Omi/HtrA2 (26) and livin [18] expression resensitizes human tumor cells to diverse proapoptotic stimuli. Anti-XIAP and anti-FLIP oligonucleotides sensitized malignant cells to the cytotoxic effects of doxorubicin, Taxol, vinorelbine, and etoposide as well as to Fas-, TNF-R-, and TRAIL-R-mediated apoptosis [40, 81]. Several groups have recently generated hammerhead ribozymes targeting human survivin mRNA and proved their efficacy by increasing apoptosis *in vitro* in prostate and breast cancer cells [16, 86]. Furthermore, human prostate cancer grafts fail to grow in athymic nude mice treated with anti-survivin ribozyme [86]. Antisense nucleotides targeting survivin have been shown to induce apoptosis or eliminate cisplatin resistance in various cell lines [14]. This approach is now exploited by Isis Pharmaceuticals (<http://www.isip.com>) and Abbott Laboratories (<http://abbott.com>) with the aim of developing clinically applicable antisense-based strategies.

Prospects and potential problems. The importance of apoptosis as a mechanism that governs the elimination of malignant cells has become evident in the past decade. The research on apoptosis revealed the key role of the caspase network as a central player in promoting programmed cell death. Since inappropriate

caspase activity or overexpression of caspase inhibitory molecules have been demonstrated in various types of cancer cells, targeting caspases or their direct modulators offers a novel cancer therapy strategy. Different approaches employing fusion proteins, cell-permeable peptides, viral vectors, antisense oligonucleotides, specific ribozymes, and siRNAs have been proposed to directly or indirectly activate caspases as well as inactivate caspase inhibitors. Very promising results obtained from studies in cell lines and animal models indicate the feasibility and efficacy of these approaches to enhance conventional chemo/radiotherapy. Since some of these approaches lack tumor selectivity, several laboratories focus their work on targeting tumor-specific antigens with monoclonal antibodies or by using gene expression systems driven by promoter elements that are frequently active in cancer cells (see above).

The near future of cancer therapy will most likely rely on the combined application of apoptosis-sensitizing strategies described above, and conventional radio- and chemotherapy. The combination of agents that activate caspases and/or inactivate caspase inhibitors with classical chemotherapeutics will be more effective than single-agent protocols. Potentiation of drug-induced apoptosis by different caspase cascade-targeting agents has been demonstrated in various malignancies including breast, prostate, colon, lung, bladder, and ovarian cancer, glioma, melanoma (see Table 4). A significant amount of attention has also been given to the combined treatment of tumor cells by caspase cascade-targeting agents and ionizing radiation. For example, ribozyme-mediated inhibition of survivin expression renders human melanoma cells more susceptible to γ -irradiation [86]. Similarly, augmentation of apoptosis in DLD-1 colon cancer cells was observed upon transfection with an adenoviral vector expressing *caspase-8* and X-ray irradiation [126].

Despite very encouraging experimental data *in vitro*, and even in animal models, several problems still await solution. Some approaches towards the regulation of caspase activity may affect apoptosis in an opposite way depending on cell type and the intensity of the applied stimulus. Examples that illustrate such an opposing effects (c-FLIP, ARC, FADD or ASC/TMS1) can be found in Table 2. Explanations of these phenomena have been provided for some molecules (see above) but not for the others. In addition, caspases and other components of the apoptotic machinery are involved in cellular processes unrelated to apoptosis, including cell activation, differentiation, survival, migration, cell-cycle progression, and maturation of cytokines [65, 88]. These often inadequately known functions of caspases may be responsible for unexpected side effects of novel, apoptotic pathway-based therapies. Furthermore, drugs may also kill cancer cells via mechanisms that do not require the activation of caspase-dependent pathways, thus the use of caspase activators may not be therapeutically relevant in such cases.

Finally, the molecules associated with caspase-dependent apoptosis should be regarded not as the

separate independent targets but as integrated and interconnected components of apoptotic signal transduction pathways. Therefore, although major apoptotic cascades have been fairly well characterized, the outcome of manipulating specific components of programmed cell death pathways may be unexpected. Thus, consequently there may be unusual side effects arising from the interference with immune response, cell differentiation and migration. Nevertheless, despite these potential shortcomings novel apoptosis-triggered drugs/approaches will fuel the development of innovative strategies in cancer treatment.

ACKNOWLEDGMENTS

We wish to acknowledge and apologize to all those authors whose work was not directly referenced here due to the space limitations. This work was in part supported by grants from the "Deutsche Krebshilfe", the DFG (LO 823/1–1 and LO 823/3–1), and IKF3 E8. M.L. is supported by CRC awarded for "New Cancer Therapy Development".

NOTE ADDED IN PROOF

After the manuscript had been accepted, Reed and co-workers reported an interesting finding of novel anti-tumor compounds which were identified from a chemical library of polyphenylureas (**Schimmer DA, et al.** Small-molecule antagonists of apoptosis suppressor XIAP exhibit broad antitumor activity. *Cancer Cell* 2004; **5**: 25–35). These nonpeptidic, small-molecule antagonists of XIAP overcome XIAP-mediated suppression of effector caspase-3 and -7 (but not apical caspase-9). According to the authors, the polyphenylurea-based XIAP antagonists not only sensitize tumor cells to apoptosis-triggering effects to a broad range of anticancer drugs but induce directly apoptosis of malignant cells *in vitro* and *in vivo* as single agents contrary to Smac peptides described in the review which are devoid of direct apoptosis-inducing activity.

REFERENCES

1. **Altieri DC.** Survivin, versatile modulation of cell division and apoptosis in cancer. *Oncogene* 2003; **22**: 8581–9.
2. Apoptosis and Cancer. Martin SJ, ed. S. Karger Publishing, 1997.
3. Apoptosis in Hormone-dependent Cancers. Tenniswood M, Michna H, eds. Ernst Schering Research Foundation Workshop, No 14. Springer Verlag, 1995.
4. **Baumler C, Duan F, Onel K, Rapaport B, Jhanwar S, Offit K, Elkon KB.** Differential recruitment of caspase 8 to cFLIP confers sensitivity or resistance to Fas-mediated apoptosis in a subset of familial lymphoma patients. *Leuk Res* 2003; **27**: 841–51.
5. **Behrendorf HA, van de Craen M, Knies UE, Vandabeele P, Clauss M.** The endothelial monocyte-activating polypeptide II (EMAP II) is a substrate for caspase-7. *FEBS Lett* 2000; **466**: 143–7.
6. **Bilim V, Kasahara T, Hara N, Takahashi K, Tomita Y.** Role of XIAP in the malignant phenotype of transitional cell cancer (TCC) and therapeutic activity of XIAP antisense oligonucleotides against multidrug-resistant TCC *in vitro*. *Int J Cancer* 2003; **103**: 29–37.
7. **Broxterman HJ, Hoekman K.** Direct activation of caspases by RGD-peptides may increase drug sensitivity of tumour cells. *Drug Resist Updat* 1999; **2**: 139–41.
8. **Buckley CD, Pilling D, Henriquez NV, Parsonage G, Threlfall K, Scheel-Toellner D, Simmons DL, Akbar AN, Lord JM, Salmon M.** RGD peptides induce apoptosis by direct caspase-3 activation. *Nature* 1999; **397**: 534–9.
9. **Bullani RR, Huard B, Viard-Leveugle I, Byers HR, Irmeler M, Saurat JH, Tschopp J, French LE.** Selective expression of FLIP in malignant melanocytic skin lesions. *J Invest Dermatol* 2001; **117**: 360–4.
10. **Caelles C, Helmbert A, Karin M.** p53-dependent apoptosis in the absence of transcriptional activation of p53-target genes. *Nature* 1994; **370**: 220–3.
11. **Cain K, Bratton SB, Cohen GM.** The Apaf-1 apoptosome: a large caspase-activating complex. *Biochimie* 2002; **84**: 203–14.
12. **Cassens U, Lewinski G, Samraj AK, von Bernuth H, Baust H, Khazaie K, Los M.** Viral modulation of cell death by inhibition of caspases. *Arch Immunol Ther Exp* 2003; **51**: 19–27.
13. **Chang DW, Ditsworth D, Liu H, Srinivasula SM, Alnemri ES, Yang X.** Oligomerization is a general mechanism for the activation of apoptosis initiator and inflammatory procaspases. *J Biol Chem* 2003; **278**: 16466–9.
14. **Chen J, Wu W, Tahir SK, Kroeger PE, Rosenberg SH, Cowsert LM, Bennett F, Krajewska S, Krajewska M, Welsh K, Reed JC, Ng SC.** Down-regulation of survivin by antisense oligonucleotides increases apoptosis, inhibits cytokinesis and anchorage-independent growth. *Neoplasia* 2000; **2**: 235–41.
15. **Cheung TH, Chung TK, Lo KW, Yu MY, Krajewski S, Reed JC, Wong YF.** Apoptosis-related proteins in cervical intraepithelial neoplasia and squamous cell carcinoma of the cervix. *Gynecol Oncol* 2002; **86**: 14–8.
16. **Choi KS, Lee TH, Jung MH.** Ribozyme-mediated cleavage of the human survivin mRNA and inhibition of antiapoptotic function of survivin in MCF-7 cells. *Cancer Gene Ther* 2003; **10**: 87–95.
17. **Clem RJ, Cheng EH, Karp CL, Kirsch DG, Ueno K, Takahashi A, Kastan MB, Griffin DE, Earnshaw WC, Veluona MA, Hardwick JM.** Modulation of cell death by Bcl-X_L through caspase interaction. *Proc Natl Acad Sci USA* 1998; **95**: 554–9.
18. **Crnkovic-Mertens I, Hoppe-Seyley F, Butz K.** Induction of apoptosis in tumor cells by siRNA-mediated silencing of the livin/ML-IAP/KIAP gene. *Oncogene* 2003; **22**: 8330–6.
19. **Degterev A, Boyce M, Yuan J.** A decade of caspases. *Oncogene* 2003; **22**: 8543–67.
20. **Devarajan E, Sahin AA, Chen JS, Krishnamurthy RR, Aggarwal N, Brun AM, Sapino A, Zhang F, Sharma D, Yang XH, Tora AD, Mehta K.** Down-regulation of caspase 3 in breast cancer: a possible mechanism for chemoresistance. *Oncogene* 2002; **21**: 8843–51.
21. **Deveraux QL, Leo E, Stennicke HR, Welsh K, Salvesen GS, Reed JC.** Cleavage of human inhibitor of apoptosis protein XIAP results in fragments with distinct specificities for caspases. *EMBO J* 1999; **18**: 5242–51.
22. **Elnemr A, Ohta T, Yachie A, Kayahara M, Kitagawa H, Fujimura T, Ninomiya I, Fushida S, Nishimura GI, Shimizu K, Miwa K.** Human pancreatic cancer cells disable function of Fas receptors at several levels in Fas signal transduction pathway. *Int J Oncol* 2001; **18**: 311–6.
23. **Enari M, Sakahira H, Yokoyama H, Okawa K, Iwamatsu A, Nagata S.** A caspase-activated DNase that

degrades DNA during apoptosis, and its inhibitor ICAD Nature 1998; **391**: 43–50.

24. **Ferri KF, Kroemer G.** Organelle-specific initiation of cell death pathways. Nat Cell Biol 2001; **3**: E255–63.

25. **French LE, Tschopp J.** Protein-based therapeutic approaches targeting death receptors. Cell Death Differ 2003; **10**: 117–23.

26. **Fu WN, Bertoni F, Kelsey SM, McElwaine SM, Cotter FE, Newland AC, Jia L.** Role of DNA methylation in the suppression of Apaf-1 protein in human leukaemia. Oncogene 2003; **22**: 451–5.

27. **Fu YG, Qu YJ, Wu KC, Zhai HH, Liu ZG, Fan DM.** Apoptosis-inducing effect of recombinant Caspase-3 expressed by constructed eukaryotic vector on gastric cancer cell line SGC7901. World J Gastroenterol 2003; **9**: 1935–9.

28. **Fulda S, Wick W, Weller M, Debatin KM.** Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma *in vivo*. Nat Med 2002; **8**: 808–15.

29. **Gonzalez-Gomez P, Bello MJ, Lomas J, Arjona D, Alonso ME, Aminoso C, Lopez-Marin I, Anselmo NP, Sarasa JL, Gutierrez M, Casartelli C, Rey JA.** Aberrant methylation of multiple genes in neuroblastic tumours. relationship with MYCN amplification and allelic status at 1p. Eur J Cancer 2003; **39**: 1478–85.

30. **Goyal L.** Cell death inhibition: keeping caspases in check. Cell 2001; **104**: 805–8.

31. **Guan X, Sagara J, Yokoyama T, Koganehira Y, Oguchi M, Saida T, Taniguchi S.** ASC/TMS1, a caspase-1 activating adaptor, is downregulated by aberrant methylation in human melanoma. Int J Cancer 2003; **107**: 202–8.

32. **Guo Y, Srinivasula SM, Druilhe A, Fernandes-Alnemri T, Alnemri ES.** Caspase-2 induces apoptosis by releasing proapoptotic proteins from mitochondria. J Biol Chem 2002; **277**: 13430–7.

33. **Hallan E, Blomhoff HK, Smeland EB, Lomo J.** Involvement of ICE (Caspase) family in gamma-radiation-induced apoptosis of normal B lymphocytes. Scand J Immunol 1997; **46**: 601–8.

34. **Hanahan D, Weinberg RA.** The hallmarks of cancer. Cell 2000; **100**: 57–70.

35. **Harada K, Toyooka S, Shivapurkar N, Maitra A, Reddy JL, Matta H, Miyajima K, Timmons CF, Tomlinson GE, Mastrangelo D, Hay RJ, Chaudhary PM, Gazdar AF.** Deregulation of caspase 8 and 10 expression in pediatric tumors and cell lines. Cancer Res 2002; **62**: 5897–901.

36. **Hirata H, Takahashi A, Kobayashi S, Yonehara S, Sawai H, Okazaki T, Yamamoto K, Sasada M.** Acinus is a caspase-3-activated protein required for apoptotic condensation. J Exp Med 1998; **187**: 587–600.

37. **Hofmann WK, de Vos S, Tsukasaki K, Wachsmann W, Pinkus GS, Said JW, Koeffler HP.** Altered apoptosis pathways in mantle cell lymphoma detected by oligonucleotide microarray. Blood 2001; **98**: 787–94.

38. **Hopkins-Donaldson S, Ziegler A, Kurtz S, Bigosch C, Kandioler D, Ludwig C, Zangemeister-Wittke U, Stahel R.** Silencing of death receptor and caspase-8 expression in small cell lung carcinoma cell lines and tumors by DNA methylation. Cell Death Differ 2003; **10**: 356–64.

39. **Hosomi Y, Gemma A, Hosoya Y, Nara M, Okano T, Takenaka K, Yoshimura A, Koizumi K, Shimizu K, Kudo S.** Somatic mutation of the Caspase-5 gene in human lung cancer. Int J Mol Med 2003; **12**: 443–6.

40. **Hu Y, Cherton-Horvat G, Dragowska V, Baird S, Korneluk RG, Durkin JP, Mayer LD, LaCasse EC.** Antisense oligonucleotides targeting XIAP induce apoptosis

and enhance chemotherapeutic activity against human lung cancer cells *in vitro* and *in vivo*. Clin Cancer Res 2003; **9**: 2826–36.

41. **Irmeler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V, Bodmer JL, Schroter M, Burns K, Mattmann C, Rimoldi D, French LE, Tschopp J.** Inhibition of death receptor signals by cellular FLIP. Nature 1997; **388**: 190–5.

42. **Izawa M, Mori T, Satoh T, Teramachi K, Sairenji T.** Identification of an alternative form of caspase-9 in human gastric cancer cell lines: a role of a caspase-9 variant in apoptosis resistance. Apoptosis 1999; **4**: 321–5.

43. **Jia LT, Zhang LH, Yu CJ, Zhao J, Xu YM, Gui JH, Jin M, Ji ZL, Wen WH, Wang CJ, Chen SY, Yang AG.** Specific tumoricidal activity of a secreted proapoptotic protein consisting of HER2 antibody and constitutively active caspase-3. Cancer Res 2003; **63**: 3257–62.

44. **Johnson DE.** Noncaspase proteases in apoptosis. Leukemia 2000; **14**: 1695–703.

45. **Johnstone RW, Ruefli AA, Lowe SW.** Apoptosis: a link between cancer genetics and chemotherapy. Cell 2002; **108**: 153–64.

46. **Kang SJ, Wang S, Hara H, Peterson EP, Namura S, Amin Hanjani S, Huang Z, Srinivasan A, Tomaselli KJ, Thornberry NA, Moskowitz MA, Yuan J.** Dual role of caspase-11 in mediating activation of caspase-1 and caspase-3 under pathological conditions. J Cell Biol 2000; **149**: 613–22.

47. **Kasof GM, Gomes BC.** Livin, a novel inhibitor of apoptosis protein family member. J Biol Chem 2001; **276**: 3238–46.

48. **Kerr JFR, Wyllie AH, Currie AR.** Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972; **26**: 239–57.

49. **Kim HS, Lee JW, Soung YH, Park WS, Kim SY, Lee JH, Park JY, Cho YG, Kim CJ, Jeong SW, Nam SW, Kim SH, Lee JY, Yoo NJ, Lee SH.** Inactivating mutations of caspase-8 gene in colorectal carcinomas. Gastroenterology 2003; **125**: 708–15.

50. **Kim PK, Park SY, Koty PP, Hua Y, Luketich JD, Billiar TR.** Fas-associating death domain protein overexpression induces apoptosis in lung cancer cells. J Thorac Cardiovasc Surg 2003; **125**: 1336–42.

51. **Kirsch DG, Doseff A, Chau BN, Lim DS, de Souza-Pinto NC, Hansford R, Kastan MB, Lazebnik YA, Hardwick JM.** Caspase-3-dependent cleavage of Bcl-2 promotes release of cytochrome c. J Biol Chem 1999; **274**: 21155–61.

52. **Kischkel FC, Hellbardt S, Behrmann I, Germer M, Pawlita M, Krammer PH, Peter ME.** Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. EMBO J 1995; **14**: 5579–88.

53. **Koga S, Hirohata S, Kondo Y, Komata T, Takakura M, Inoue M, Kyo S, Kondo S.** FADD gene therapy using the human telomerase catalytic subunit (hTERT) gene promoter to restrict induction of apoptosis to tumors *in vitro* and *in vivo*. Anticancer Res 2001; **21**: 1937–43.

54. **Kolenko V, Uzzo RG, Bukowski R, Bander NH, Novick AC, Hsi ED, Finke JH.** Dead or dying: necrosis versus apoptosis in caspase-deficient human renal cell carcinoma. Cancer Res 1999; **59**: 2838–42.

55. **Komata T, Kondo Y, Kanzawa T, Hirohata S, Koga S, Sumiyoshi H, Srinivasula SM, Barna BP, Germano IM, Takakura M, Inoue M, Alnemri ES, Shay JW, Kyo S, Kondo S.** Treatment of malignant glioma cells

with the transfer of constitutively active caspase-6 using the human telomerase catalytic subunit (human telomerase reverse transcriptase) gene promoter. *Cancer Res* 2001; **61**: 5796–802.

56. **Komata T, Kondo Y, Kanzawa T, Ito H, Hirohata S, Koga S, Sumiyoshi H, Takakura M, Inoue M, Barina BP, Germano IM, Kyo S, Kondo S.** Caspase-8 gene therapy using the human telomerase reverse transcriptase promoter for malignant glioma cells. *Hum Gene Ther* 2002; **13**: 1015–25.

57. **Koseki T, Inohara N, Chen S, Nunez G.** ARC, an inhibitor of apoptosis expressed in skeletal muscle and heart that interacts selectively with caspases. *Proc Natl Acad Sci USA* 1998; **95**: 5156–60.

58. **Kumamoto H, Kimi K, Ooya K.** Immunohistochemical analysis of apoptosis-related factors (Fas, Fas ligand, caspase-3 and single-stranded DNA) in ameloblastomas. *J Oral Pathol Med* 2001; **30**: 596–602.

59. **Liang Y, Yan C, Schor NF.** Apoptosis in the absence of caspase 3. *Oncogene* 2001; **20**: 6570–8.

60. **Liu B, Peng D, Lu Y, Jin W, Fan Z.** A novel single amino acid deletion caspase-8 mutant in cancer cells that lost proapoptotic activity. *J Biol Chem* 2002; **277**: 30159–64.

61. **Liu JR, Opipari AW, Tan L, Jiang Y, Zhang Y, Tang H, Nunez G.** Dysfunctional apoptosome activation in ovarian cancer: implications for chemoresistance. *Cancer Res* 2002; **62**: 924–31.

62. **Liu LX, Liu ZH, Jiang HC, Qu X, Zhang WH, Wu LF, Zhu AL, Wang XQ, Wu M.** Profiling of differentially expressed genes in human gastric carcinoma by cDNA expression array. *World J Gastroenterol* 2002; **8**: 580–5.

63. **Los M, Burek CJ, Stroh C, Benedyk K, Hug H, Mackiewicz A.** Anticancer drugs of tomorrow: apoptotic pathways as targets for drug design. *Drug Discov Today* 2003; **8**: 67–77.

64. **Los M, Mozoluk M, Ferrari D, Stepczynska A, Stroh C, Renz A, Herczeg Z, Wang ZQ, Schulze-Osthoff K.** Activation and caspase-mediated inhibition of PARP: a molecular switch between fibroblast necrosis and apoptosis in death receptor signaling. *Mol Biol Cell* 2002; **13**: 978–88.

65. **Los M, Stroh C, Janicke RU, Engels IH, Schulze-Osthoff K.** Caspases: more than just killers? *Trends Immunol* 2001; **22**: 31–4.

66. **Los M, Van de Craen M, Penning LC, Schenk H, Westendorp M, Baeuerle PA, Droge W, Krammer PH, Fiers W, Schulze-Osthoff K.** Requirement of an ICE/CED-3 protease for Fas/APO-1-mediated apoptosis. *Nature* 1995; **375**: 81–3.

67. **Mancini M, Machamer CE, Roy S, Nicholson DW, Thornberry NA, Casciola-Rosen LA, Rosen A.** Caspase-2 is localized at the Golgi complex and cleaves golgin-160 during apoptosis. *J Cell Biol* 2000; **149**: 603–12.

68. **Mandrizzato S, Brasseur F, Andry G, Boon T, van der Bruggen P.** A CASP-8 mutation recognized by cytolytic T lymphocytes on a human head and neck carcinoma. *J Exp Med* 1997; **186**: 785–93.

69. **Martins LM, Iaccarino I, Tenev T, Gschmeissner S, Totty NF, Lemoine NR, Savopoulos J, Gray CW, Creasy CL, Dingwall C, Downward J.** The serine protease Omi/HtrA2 regulates apoptosis by binding XIAP through a reaper-like motif. *J Biol Chem* 2002; **277**: 439–44.

70. **McCarthy JV, Ni J, Dixit VM.** RIP2 is a novel NF-kappaB-activating and cell death-inducing kinase. *J Biol Chem* 1998; **273**: 16968–75.

71. **McKay TR, Bell S, Tenev T, Stoll V, Lopes R, Lemoine NR, McNeish IA.** Procaspase 3 expression in ovarian carcinoma cells increases survivin transcription which can be countered with a dominant-negative mutant, survivin T34A; a combination gene therapy strategy. *Oncogene* 2003; **22**: 3539–47.

72. **McNeish IA, Bell S, McKay T, Tenev T, Marani M, Lemoine NR.** Expression of Smac/DIABLO in ovarian carcinoma cells induces apoptosis via a caspase-9-mediated pathway. *Exp Cell Res* 2003; **286**: 186–98.

73. **Mesri M, Wall NR, Li J, Kim RW, Altieri DC.** Cancer gene therapy using a survivin mutant adenovirus. *J Clin Invest* 2001; **108**: 981–90.

74. **Mishima K, Nariai Y, Yoshimura Y.** Carboplatin induces Fas (APO-1/CD95)-dependent apoptosis of human tongue carcinoma cells: sensitization for apoptosis by upregulation of FADD expression. *Int J Cancer* 2003; **105**: 593–600.

75. **Monks A, Andreeff M, Reed JC.** Expression and prognostic significance of IAP-family genes in human cancers and myeloid leukemias. *Clin Cancer Res* 2000; **6**: 1796–803.

76. **Moriai R, Tsuji N, Kobayashi D, Yagihashi A, Namiki Y, Takahashi H, Watanabe N.** A proapoptotic caspase recruitment domain protein gene, TMS1, is hypermethylated in human breast and gastric cancers. *Anticancer Res* 2002; **22**: 4163–8.

77. **Mueller T, Voigt W, Simon H, Fruehauf A, Bulankin A, Grothey A, Schmol HJ.** Failure of activation of caspase-9 induces a higher threshold for apoptosis and cisplatin resistance in testicular cancer. *Cancer Res* 2003; **63**: 513–21.

78. **Narayan G, Pulido HA, Koul S, Lu XY, Harris CP, Yeh YA, Vargas H, Posso H, Terry MB, Gissmann L, Schneider A, Mansukhani M, Rao PH, Murty VV.** Genetic analysis identifies putative tumor suppressor sites at 2q35-q36.1 and 2q36.3-q37.1 involved in cervical cancer progression. *Oncogene* 2003; **22**: 3489–99.

79. **Newton K, Strasser A.** Cell death control in lymphocytes. *Adv Immunol* 2000; **76**: 179–226.

80. **Nor JE, Hu Y, Song W, Spencer DM, Nunez G.** Ablation of microvessels *in vivo* upon dimerization of iCaspase-9. *Gene Ther* 2002; **9**: 444–51.

81. **Okano H, Shiraki K, Inoue H, Kawakita T, Saitou Y, Enokimura N, Yamamoto N, Sugimoto K, Fujikawa K, Murata K, Nakano T.** Over-expression of Smac promotes TRAIL-induced cell death in human hepatocellular carcinoma. *Int J Mol Med* 2003; **12**: 25–8.

82. **Okano H, Shiraki K, Inoue H, Kawakita T, Yamanaka T, Deguchi M, Sugimoto K, Sakai T, Ohmori S, Fujikawa K, Murata K, Nakano T.** Cellular FLICE/caspase-8-inhibitory protein as a principal regulator of cell death and survival in human hepatocellular carcinoma. *Lab Invest* 2003; **83**: 1033–43.

83. **Paggi MG, Catricala C, Amantea A, Picardo M, Natali PG, Baldi F, Baldi A.** SP-22 analysis of APAF-1 expression in human cutaneous melanoma progression. *Pigment Cell Res* 2003; **16**: 589.

84. **Palmerini F, Devilard E, Jarry A, Birg F, Xerri L.** Caspase 7 downregulation as an immunohistochemical marker of colonic carcinoma. *Hum Pathol* 2001; **32**: 461–7.

85. **Park WS, Lee JH, Shin MS, Park JY, Kim HS, Lee JH, Kim YS, Lee SN, Xiao W, Park CH, Lee SH, Yoo NJ, Lee JY.** Inactivating mutations of the caspase-10 gene in gastric cancer. *Oncogene* 2002; **21**: 2919–25.

86. **Pennati M, Binda M, Colella G, Folini M, Citti L, Villa R, Daidone MG, Zaffaroni N.** Radiosensitization of

human melanoma cells by ribozyme-mediated inhibition of survivin expression. *J Invest Dermatol* 2003; **120**: 648–54.

87. **Pennati M, Binda M, Colella G, Zoppe M, Folini M, Vignati S, Valentini A, Citti L, De Cesare M, Pratesi G, Giacca M, Daidone MG, Zaffaroni N.** Ribozyme-mediated inhibition of survivin expression increases spontaneous and drug-induced apoptosis and decreases the tumorigenic potential of human prostate cancer cells. *Oncogene* 2004; **23**: 386–94.

88. **Philchenkov AA.** Caspases as regulators of apoptosis and other cell functions. *Biochemistry (Mosc)* 2003; **68**: 365–76.

89. **Philchenkov AA, Butenko ZA.** The mechanisms of apoptosis regulation and the antiapoptotic action of oncogenic viruses. *Biopolymers & Cell* 2000; **16**: 122–35.

90. **Philchenkov AA, Stoika RS.** Apoptosis and Cancer: Moving from Theory to Clinics. *Ukrmedknyha*, 2004.

91. **Prokop A, Wieder T, Sturm I, Essmann F, Seeger K, Wuchter C, Ludwig WD, Henze G, Dorken B, Daniel PT.** Relapse in childhood acute lymphoblastic leukemia is associated with a decrease of the Bax/Bcl-2 ratio and loss of spontaneous caspase-3 processing *in vivo*. *Leukemia* 2000; **14**: 1606–13.

92. **Rao RV, Ellerby HM, Bredesen DE.** Coupling endoplasmic reticulum stress to the cell death program. *Cell Death Differ* 2004; **11**: 372–80.

93. **Richter BW, Mir SS, Eiben LJ, Lewis J, Refey SB, Frattini A, Tian L, Frank S, Youle RJ, Nelson DL, Notarangelo LD, Vezoni P, Fearnhead HO, Duckett CS.** Molecular cloning of ILP-2, a novel member of the inhibitor of apoptosis protein family. *Mol Cell Biol* 2001; **21**: 4292–301.

94. **Roberg K.** Relocalization of cathepsin D and cytochrome *c* early in apoptosis revealed by immunoelectron microscopy. *Lab Invest* 2001; **81**: 149–58.

95. **Roberg K, Kagedal K, Ollinger K.** Microinjection of cathepsin D induces caspase-dependent apoptosis in fibroblasts. *Am J Pathol* 2002; **161**: 89–96.

96. **Rosen A, Casciola-Rosen L.** Macromolecular substrates for the ICE-like proteases during apoptosis. *J Cell Biochem* 1997; **64**: 50–4.

97. **Roy N, Deveraux QL, Takahashi R, Salvesen GS, Reed JC.** The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. *EMBO J* 1997; **16**: 6914–25.

98. **Roy S, Bayly CI, Gareau Y, Houtzager VM, Kargman S, Keen SL, Rowland K, Seiden IM, Thornberry NA, Nicholson DW.** Maintenance of caspase-3 proenzyme dormancy by an intrinsic “safety catch” regulatory tripeptide. *Proc Natl Acad Sci USA* 2001; **98**: 6132–7.

99. **Ryu BK, Lee MG, Chi SG, Kim YW, Park JH.** Increased expression of cFLIP(L) in colonic adenocarcinoma. *J Pathol* 2001; **194**: 15–9.

100. **Sadowski-Debbing K, Coy JF, Mier W, Hug H, Los M.** Caspases — their role in apoptosis and other physiological processes as revealed by knock-out studies. *Arch Immunol Ther Exp* 2002; **50**: 19–34

101. **Sahara S, Aoto M, Eguchi Y, Imamoto N, Yoneda Y, Tsujimoto Y.** Acinus is a caspase-3-activated protein required for apoptotic chromatin condensation. *Nature* 1999; **401**: 168–73.

102. **Scaffidi C, Schmitz I, Krammer PH, Peter ME.** The role of c-FLIP in modulation of CD95-induced apoptosis. *J Biol Chem* 1999; **274**: 1541–8.

103. **Schimmer AD, Pedersen IM, Kitada S, Eksioğlu-Demiralp E, Minden MD, Pinto R, Mah K, Andreff M, Kim Y, Suh WS, Reed JC.** Functional blocks in

caspase activation pathways are common in leukemia and predict patient response to induction chemotherapy. *Cancer Res* 2003; **63**: 1242–8.

104. **Schwartz S Jr, Yamamoto H, Navarro M, Maestro M, Reventos J, Perucho M.** Frameshift mutations at mononucleotide repeats in caspase-5 and other target genes in endometrial and gastrointestinal cancer of the microsatellite mutator phenotype. *Cancer Res* 1999; **59**: 2995–3002.

105. **Scott S, Kimura T, Ichinohasama R, Bergen S, Magliocco A, Reimer C, Kerviche A, Sheridan D, Decoteau JF.** Microsatellite mutations of transforming growth factor-beta receptor type II and caspase-5 occur in human precursor T-cell lymphoblastic lymphomas/leukemias *in vivo* but are not associated with hMSH2 or hMLH1 promoter methylation. *Leuk Res* 2003; **27**: 23–34.

106. **Shariat SF, Desai S, Song W, Khan T, Zhao J, Nguyen C, Foster BA, Greenberg N, Spencer DM, Slawin KM.** Adenovirus-mediated transfer of inducible caspases: a novel “death switch” gene therapeutic approach to prostate cancer. *Cancer Res* 2001; **61**: 2562–71.

107. **Shin MS, Kim HS, Kang CS, Park WS, Kim SY, Lee SN, Lee JH, Park JY, Jang JJ, Kim CW, Kim SH, Lee JY, Yoo NJ, Lee SH.** Inactivating mutations of CASP10 gene in non-Hodgkin lymphomas. *Blood* 2002; **99**: 4094–9.

108. **Shin MS, Kim HS, Lee SH, Lee JW, Song YH, Kim YS, Park WS, Kim SY, Lee SN, Park JY, Lee JH, Xiao W, Jo KH, Wang YP, Lee KY, Park YG, Kim SH, Lee JY, Yoo NJ.** Alterations of Fas-pathway genes associated with nodal metastasis in non-small cell lung cancer. *Oncogene* 2002; **21**: 4129–36.

109. **Shinoura N, Sakurai S, Asai A, Kirino T, Hamada H.** Co-transduction of Apaf-1 and caspase-9 augments etoposide-induced apoptosis in U-373MG glioma cells. *Jpn J Cancer Res* 2001; **92**: 467–74.

110. **Shivapurkar N, Reddy J, Matta H, Sathyanarayana UG, Huang CX, Toyooka S, Minna JD, Chaudhary PM, Gazdar AF.** Loss of expression of death-inducing signaling complex (DISC) components in lung cancer cell lines and the influence of MYC amplification. *Oncogene* 2002; **21**: 8510–4.

111. **Siegmund D, Hadwiger P, Pfizenmaier K, Vornlocher HP, Wajant H.** Selective inhibition of FLICE-like inhibitory protein expression with small interfering RNA oligonucleotides is sufficient to sensitize tumor cells for TRAIL-induced apoptosis. *Mol Med* 2002; **8**: 725–32.

112. **Slee EA, Harte MT, Kluck RM, Wolf BB, Casiano CA, Newmeyer DD, Wang HG, Reed JC, Nicholson DW, Alnemri ES, Green DR, Martin SJ.** Ordering the cytochrome *c*-initiated caspase cascade: hierarchical activation of caspases-2, -3, -6, -7, -8, and -10 in a caspase-9-dependent manner. *J Cell Biol* 1999; **144**: 281–92.

113. **Soengas MS, Capodiceci P, Polsky D, Mora J, Esteller M, Opitz-Araya X, McCombie R, Herman JG, Gerald WL, Lazebnik YA, Cordon-Cardo C, Lowe SW.** Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. *Nature* 2001; **409**: 207–11.

114. **Sohn JH, Kim DH, Choi NG, Park YE, Ro JY.** Caspase-3/CPP32 immunoreactivity and its correlation with frequency of apoptotic bodies in human prostatic carcinomas and benign nodular hyperplasias. *Histopathology* 2000; **37**: 555–60.

115. **Soung YH, Lee JW, Kim HS, Park WS, Kim SY, Lee JH, Park JY, Cho YG, Kim CJ, Park YG, Nam SW, Jeong SW, Kim SH, Lee JY, Yoo NJ, Lee SH.** Inactivating mutations of CASPASE-7 gene in human cancers. *Oncogene* 2003; **22**: 6104–8.

116. **Stroh C, Cassens U, Samraj AK, Sibrowski W, Schulze-Osthoff K, Los M.** The role of caspases in cryoinjury: caspase inhibition strongly improves the recovery of cryopreserved hematopoietic and other cells. *FASEB J* 2002; **16**: 1651–3.
117. **Sun XM, MacFarlane M, Zhuang J, Wolf BB, Green DR, Cohen GM.** Distinct caspase cascades are initiated in receptor-mediated and chemical-induced apoptosis. *J Biol Chem* 1999; **274**: 5053–60.
118. **Takahashi R, Deveraux Q, Tamm I, Welsh K, AssaMunt N, Salvesen GS, Reed JC.** A single BIR domain of XIAP sufficient for inhibiting caspases. *J Biol Chem* 1998; **273**: 7787–90.
119. **Takeuchi S, Takeuchi N, Fermin AC, Taguchi H, Koeffler HP.** Frameshift mutations in caspase-5 and other target genes in leukemia and lymphoma cell lines having microsatellite instability. *Leuk Res* 2003; **27**: 359–61.
120. **Takita J, Yang HW, Chen YY, Hanada R, Yamamoto K, Teitz T, Kidd V, Hayashi Y.** Allelic imbalance on chromosome 2q and alterations of the caspase 8 gene in neuroblastoma. *Oncogene* 2001; **20**: 4424–32.
121. **Teitz T, Wei T, Valentine MB, Vanin EF, Grenet J, Valentine VA, Behm FG, Look AT, Lahti JM, Kidd VJ.** Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. *Nat Med* 2000; **6**: 529–35.
122. **Thomas RK, Kallenborn A, Wickenhauser C, Schultze JL, Draube A, Vockerodt M, Re D, Diehl V, Wolf J.** Constitutive expression of c-FLIP in Hodgkin and Reed-Sternberg cells. *Am J Pathol* 2002; **160**: 1521–8.
123. **Thomas SB.** Apoptosis and Cell Cycle Control in Cancer: Basic Mechanisms and Implications for Treating Malignant Disease. Bios Scientific Publishers, 1996.
124. **Tourneur L, Mistou S, Michiels FM, Devauchelle V, Renia L, Feunteun J, Chiocchia G.** Loss of FADD protein expression results in a biased Fas-signaling pathway and correlates with the development of tumoral status in thyroid follicular cells. *Oncogene* 2003; **22**: 2795–804.
125. **Tse E, Rabbitts TH.** Intracellular antibody-caspase-mediated cell killing: an approach for application in cancer therapy. *Proc Natl Acad Sci USA* 2000; **97**: 12266–71.
126. **Uchida H, Shinoura N, Kitayama J, Watanabe T, Nagawa H, Hamada H.** Caspase-8 gene transduction augments radiation-induced apoptosis in DLD-1 cells. *Biochem Biophys Res Commun* 2002; **292**: 347–54.
127. **Uchida H, Shinoura N, Kitayama J, Watanabe T, Nagawa H, Hamada H.** 5-Fluorouracil efficiently enhanced apoptosis induced by adenovirus-mediated transfer of caspase-8 in DLD-1 colon cancer cells. *J Gene Med* 2003; **5**: 287–99.
128. **Van de Craen M, Declercq W, van den Brande I, Fiers W, Vandenabeele P.** The proteolytic procaspase activation network: an *in vitro* analysis. *Cell Death Differ* 1999; **6**: 1117–24.
129. **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; **102**: 43–53.
130. **Vincenz C.** Death receptors and apoptosis. Deadly signaling and evasive tactics. *Cardiol Clin* 2001; **19**: 31–43.
131. **Virmani A, Rathi A, Sugio K, Sathyanarayana UG, Toyooka S, Kischel FC, Tonk V, Padar A, Takahashi T, Roth JA, Euhus DM, Minna JD, Gazdar AF.** Aberrant methylation of TMS1 in small cell, non small cell lung cancer and breast cancer. *Int J Cancer* 2003; **106**: 198–204.
132. **Widmann C, Gibson S, Johnson GL.** Caspase-dependent cleavage of signaling proteins during apoptosis. A turn-off mechanism for anti-apoptotic signals. *J Biol Chem* 1998; **273**: 7141–7.
133. **Williams NS, Gaynor RB, Scoggin S, Verma U, Gokaslan T, Simmang C, Fleming J, Tavara D, Frenkel E, Becerra C.** Identification and validation of genes involved in the pathogenesis of colorectal cancer using cDNA microarrays and RNA interference. *Clin Cancer Res* 2003; **9**: 931–46.
134. **Winter RN, Kramer A, Borkowski A, Kyprianou N.** Loss of caspase-1 and caspase-3 protein expression in human prostate cancer. *Cancer Res* 2001; **61**: 1227–32.
135. **Wolf BB, Green DR.** Suicidal tendencies: apoptotic cell death by caspase family proteinases. *J Biol Chem* 1999; **274**: 20049–52.
136. **Wolf BB, Schuler M, Li W, Eggers-Sedlet B, Lee W, Tailor P, Fitzgerald P, Mills GB, Green DR.** Defective cytochrome *c*-dependent caspase activation in ovarian cancer cell lines due to diminished or absent apoptotic protease activating factor-1 activity. *J Biol Chem* 2001; **276**: 34244–51.
137. **Wu G, Chai J, Suber TL, Wu JW, Du C, Wang X, Shi Y.** Structural basis of IAP recognition by Smac/DIABLO. *Nature* 2000; **408**: 1008–12.
138. **Xia C, Xu Z, Yuan X, Uematsu K, You L, Li K, Li L, McCormick F, Jablons DM.** Induction of apoptosis in mesothelioma cells by antisurvivin oligonucleotides. *Mol Cancer Ther* 2002; **1**: 687–94.
139. **Xie X, Zhao X, Liu Y, Zhang J, Matusik RJ, Slawin KM, Spencer DM.** Adenovirus-mediated tissue-targeted expression of a caspase-9-based artificial death switch for the treatment of prostate cancer. *Cancer Res* 2001; **61**: 6795–804.
140. **Yang L, Mashima T, Sato S, Mochizuki M, Sakamoto H, Yamori T, Oh-Hara T, Tsuruo T.** Predominant suppression of apoptosome by inhibitor of apoptosis protein in non-small cell lung cancer H460 cells: therapeutic effect of a novel polyarginine-conjugated Smac peptide. *Cancer Res* 2003; **63**: 831–7.
141. **Yang QH, Church-Hajduk R, Ren J, Newton ML, Du C.** Omi/HtrA2 catalytic cleavage of inhibitor of apoptosis (IAP) irreversibly inactivates IAPs and facilitates caspase activity in apoptosis. *Genes Dev* 2003; **17**: 1487–96.
142. **Yoo NJ, Kim HS, Kim SY, Park WS, Park CH, Jeon HM, Jung ES, Lee JY, Lee SH.** Immunohistochemical analysis of Smac/DIABLO expression in human carcinomas and sarcomas. *APMIS* 2003; **111**: 382–8.
143. **Yu J, Ni M, Xu J, Zhang H, Gao B, Gu J, Chen J, Zhang L, Wu M, Zhen S, Zhu J.** Methylation profiling of twenty promoter-CpG islands of genes which may contribute to hepatocellular carcinogenesis. *BMC Cancer* 2002; **2**: 29.
144. **Zhang H, Xu Q, Krajewski S, Krajewska M, Xie Z, Fuess S, Kitada S, Godzik A, Reed JC.** BAR: An apoptosis regulator at the intersection of caspases and Bcl-2 family proteins. *Proc Natl Acad Sci USA* 2000; **97**: 2597–602.
145. **Zheng TS, Hunot S, Kuida K, Momoi T, Srinivasan A, Nicholson DW, Lazebnik Y, Flavell RA.** Deficiency in caspase-9 or caspase-3 induces compensatory caspase activation. *Nat Med* 2000; **6**: 1241–7.

КАСПАЗЫ И РАК: МЕХАНИЗМЫ ИНАКТИВАЦИИ И НОВЫЕ ПОДХОДЫ К ТЕРАПИИ

Удаление избыточных или мутировавших соматических клеток в организме осуществляется с помощью различных механизмов, включая апоптоз. Нарушение путей передачи апоптотического сигнала и реализации апоптоза может играть определенную роль в онкогенезе. Ключевыми эффекторными молекулами апоптоза являются специфические протеазы, расщепляющие белки по остаткам цистеина. Они получили название каспаз. В обзоре рассматриваются различные нарушения каспазо-зависимых механизмов реализации клеточной гибели, выявляемые в опухолевых клетках. К числу таких нарушений относятся не только мутации в генах каспаз, но и изменения степени метилирования их генов, а также нарушения стабильности соответствующих мРНК. В обзоре рассмотрены различные молекулы, участвующие в реализации апоптоза, индуцированного внешними факторами, такие, как CD95 (APO-1/Fas), FADD, FLIP, FLICE, другие апикальные каспазы, а также компоненты митохондриального пути активации апоптоза — Araf-1 и каспаза-9. Рассмотрены также эндогенные модуляторы апоптоза, такие, как IAPs, Smac/DIABLO, OMI/HtrA2 и другие белки, участвующие в регуляции апоптоза. Приводятся новейшие данные о веществах, обладающих направленным действием по отношению к тем или иным эффекторным звеньям апоптоза, которые могут оказаться перспективными средствами противоопухолевой терапии. Особое внимание уделяется перспективам комбинированного применения средств, воздействующих на компоненты передачи апоптотических сигналов, и классических методов противоопухолевой терапии.

Ключевые слова: апоптоз, каспаза, мутация, опухолевая клетка, раковые заболевания, активатор каспаз, химерный белок, пептид с высокой клеточной проницаемостью, противоопухолевые препараты, генная терапия, интерферирующая РНК, доклинические испытания.