Apoptosis is an energy-dependent, tightly regulated and selective physiologic process that governs the removal of superfluous or mutated somatic 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) absence 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
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 benzoyloxycarbonyl–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 aspatic 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–1β maturation may

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

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Size of enzyme precursor (kDa)</th>
<th>Predomain type</th>
<th>Active subunits (kDa)</th>
<th>Activation adaptor</th>
<th>Recognized subunit sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Apoptosis-initiating caspases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspase–2</td>
<td>51</td>
<td>Long, with CARD region</td>
<td>19/12</td>
<td>RAADD, PAPAC, DEFCAP</td>
<td>DEHD</td>
</tr>
<tr>
<td>Caspase–8</td>
<td>55</td>
<td>Long, with two DED–regions</td>
<td>19/11</td>
<td>FADD, DEDAF, ASC</td>
<td>LETD</td>
</tr>
<tr>
<td>Caspase–9</td>
<td>45</td>
<td>Long, with CARD region</td>
<td>17/12</td>
<td>Apf-1, Nod-1, PACAP</td>
<td>LEH</td>
</tr>
<tr>
<td>Caspase–10</td>
<td>55</td>
<td>Long, with two DED–regions</td>
<td>17/12</td>
<td>FADD, DEDAF</td>
<td>Unknown</td>
</tr>
<tr>
<td>Caspase–12</td>
<td>50</td>
<td>Long, with CARD region</td>
<td>20/10</td>
<td>TRAF-2</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>Effector caspases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspase–3</td>
<td>32</td>
<td>Short</td>
<td>17/12</td>
<td>NA</td>
<td>DEVD</td>
</tr>
<tr>
<td>Caspase–6</td>
<td>34</td>
<td>Short</td>
<td>19/11</td>
<td>NA</td>
<td>VEHD</td>
</tr>
<tr>
<td>Caspase–7</td>
<td>35</td>
<td>Short</td>
<td>20/12</td>
<td>NA</td>
<td>DEVD</td>
</tr>
<tr>
<td><strong>Caspases involved in inflammation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caspase–1</td>
<td>46</td>
<td>Long, with CARD region</td>
<td>20/10</td>
<td>CARD, ASC, CARD-8, Ipaf, Nod-1</td>
<td>WHED</td>
</tr>
<tr>
<td>Caspase–4</td>
<td>43</td>
<td>Long, with CARD region</td>
<td>20/10</td>
<td>Unknown</td>
<td>(WL)EH</td>
</tr>
<tr>
<td>Caspase–5</td>
<td>48</td>
<td>Long, with CARD region</td>
<td>20/10</td>
<td>Unknown</td>
<td>(WL)EH</td>
</tr>
<tr>
<td>Caspase–11*</td>
<td>42</td>
<td>Long, with CARD region</td>
<td>20/10</td>
<td>Unknown</td>
<td>(IL/VP)EH</td>
</tr>
<tr>
<td><strong>Other mammalian caspases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Caspase–14</td>
<td>30</td>
<td>Short</td>
<td>20/10</td>
<td>NA</td>
<td>Unknown</td>
</tr>
<tr>
<td><strong>Invertebrate caspases</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co-3</td>
<td>56</td>
<td>Long, with CARD region</td>
<td>17/14</td>
<td>Ced–4</td>
<td>DEVD</td>
</tr>
<tr>
<td>Dop-1*</td>
<td>56</td>
<td>Short</td>
<td>22/13</td>
<td>NA</td>
<td>DEVD</td>
</tr>
<tr>
<td>Drosp</td>
<td>50</td>
<td>Long, with CARD region</td>
<td>20/14</td>
<td>DARK</td>
<td>VENAD</td>
</tr>
</tbody>
</table>

* Adapted from Ref. 135 with modifications.

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

* NA — not applicable.

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

* Detected in murine cells.

* Only several caspases are given as an example.

* The *Drosophila* caspases.

* 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 sevenzymogens 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–1β–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 pi), 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, Ipaf, 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.
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, necrostatin–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 Ca\(^{2+}\) content or inducing of ER stress (changes in Ca\(^{2+}\) 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 golgin–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 act–
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-α, 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, 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 CARDI AK/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--1β, 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 (F LIC E 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 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–xL, 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 multimerization 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 tissue 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, 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**

<table>
<thead>
<tr>
<th>Protein and/or gene</th>
<th>Pro– or anti–apoptotic activity</th>
<th>Tissue</th>
<th>Type of change in expression</th>
<th>Cancer</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspase–1</td>
<td>Proapoptotic</td>
<td>Decreased</td>
<td>Prostate cancer; colon cancer</td>
<td>[134]</td>
<td></td>
</tr>
<tr>
<td>Caspase–2</td>
<td>Proapoptotic</td>
<td>Decreased</td>
<td>Mantle cell lymphoma</td>
<td>[37]</td>
<td></td>
</tr>
<tr>
<td>Caspase–3</td>
<td>Proapoptotic</td>
<td>Decreased</td>
<td>Breast cancer; RCC; prostate carcinoma; cervical carcinoma; basal cell ameloblastomas; relapse in childhood ALL</td>
<td>[15, 20, 54, 58, 84, 91, 110, 121, 134]</td>
<td></td>
</tr>
<tr>
<td>Caspase–6</td>
<td>Proapoptotic</td>
<td>Decreased</td>
<td>Cervical squamous cell carcinoma</td>
<td>[15]</td>
<td></td>
</tr>
<tr>
<td>Caspase–7</td>
<td>Proapoptotic</td>
<td>Decreased</td>
<td>Colon cancer</td>
<td>[84]</td>
<td></td>
</tr>
<tr>
<td>Caspase–8</td>
<td>Proapoptotic</td>
<td>Decreased</td>
<td>Childhood neuroblastomas; RCC; SCLC; familial lymphoma patients</td>
<td>[4, 54, 110, 121]</td>
<td></td>
</tr>
<tr>
<td>Caspase–9</td>
<td>Proapoptotic</td>
<td>Decreased</td>
<td>Colon cancer</td>
<td>[84]</td>
<td></td>
</tr>
<tr>
<td>Caspase–10</td>
<td>Proapoptotic</td>
<td>Decreased</td>
<td>Cervical carcinoma; gastric carcinoma; RCC; SCLC; NSCLC</td>
<td>[54, 62, 78, 110]</td>
<td></td>
</tr>
<tr>
<td>Apaf–1</td>
<td>Proapoptotic</td>
<td>Decreased</td>
<td>Malignant melanoma; ovarian cancer</td>
<td>[83, 113, 130]</td>
<td></td>
</tr>
<tr>
<td>FADD</td>
<td>Proapoptotic</td>
<td>Decreased</td>
<td>Thyroid carcinoma; tongue carcinoma; mantle cell lymphoma</td>
<td>[37, 74, 124]</td>
<td></td>
</tr>
<tr>
<td>Smac</td>
<td>Proapoptotic</td>
<td>Decreased</td>
<td>Lung and prostate carcinomas; malignant schwannomas; rhabdomyosarcoma; malignant fibrohistiocytoma; kidney tumors; angiosarcoma; liposarcoma; hematocarcinoma</td>
<td>[81, 142]</td>
<td></td>
</tr>
<tr>
<td>ASC/TMS1</td>
<td>Proapoptotic</td>
<td>Decreased</td>
<td>Breast cancer; gastric cancer; melanoma; lung cancer</td>
<td>[31, 76, 131]</td>
<td></td>
</tr>
<tr>
<td>XIAP</td>
<td>Antiapoptotic</td>
<td>Increased</td>
<td>NSCLC; transitional cell carcinoma of the upper urinary tract; AML</td>
<td>[6, 75, 140]</td>
<td></td>
</tr>
<tr>
<td>Survivin</td>
<td>Antiapoptotic</td>
<td>Increased</td>
<td>Tumors of lung, breast, colon, stomach, esophagus, pancreas, liver, uterus, ovary, neuroblastoma; pheochromocytoma; soft–tissue sarcoma; brain tumors; melanoma; Hodgkin’s disease; non–Hodgkin’s lymphoma; leukemias; MDSRA</td>
<td>[1]</td>
<td></td>
</tr>
<tr>
<td>c–FLIP</td>
<td>Antiapoptotic</td>
<td>Increased</td>
<td>Hepatocellular carcinoma; SCLC; pancreatic cancer; malignant melanoma; colon carcinoma; familial lymphoma patients; Hodgkin’s disease</td>
<td>[4, 9, 22, 82, 99, 110, 122]</td>
<td></td>
</tr>
</tbody>
</table>

* 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 polymorphic 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 TGFβ–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 prodomain 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 hypermethylation is involved in the inactivation of caspase-8. The aberrant hypermethylation of 11 genes, including caspase-8, was shown in a series of 44 neuroblastoma 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 down-regulation 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–xL, 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-
logical 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 autoamplifying 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 coworkers [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 anti–body–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(R8) 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 agents 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–competent 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–
Another promising strategy in gene therapy of cancer appears to have potential as a novel therapy for cancer when coupled with cancer selective targeting techniques.

<table>
<thead>
<tr>
<th>No</th>
<th>Target</th>
<th>Target-based agents</th>
<th>In vitro results</th>
<th>In vivo results</th>
<th>References</th>
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<td>1</td>
<td>Caspase-3, caspase-9</td>
<td>Replication-deficient adenoviral vectors Ad-G/iCasp3 or Ad-G/iCasp1 or Ad-G/iCasp+ CID</td>
<td>Induction of apoptosis in human prostate cancer LNCaP and PC-3 cells</td>
<td>Inhibition of growth and decrease in volume of TRAMP-C2 tumors</td>
<td>[106]</td>
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<td>2</td>
<td>Caspase-3</td>
<td>Eukaryotic expression vector pDNA-Rev-Caspase-3</td>
<td>Decrease in growth and induction of apoptosis in gastric cancer SGC7901 cells</td>
<td>—</td>
<td>[27]</td>
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<tr>
<td>3</td>
<td>Vector expressing chimeric immunocaspase-3 gene</td>
<td>Selective death of tumor cells overexpressing HER2</td>
<td>Tumor regression in a mouse HER2-positive xenografts</td>
<td>—</td>
<td>[43]</td>
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<tr>
<td>4</td>
<td>ScFv–caspase-3 fusion protein</td>
<td>Selective killing of Chinese hamster ovary CHO cells</td>
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<td>5</td>
<td>Procaspe-3 and survivin</td>
<td>Adenoviral vectors Ad-Caspase-3 + Ad-HTRT in T34A</td>
<td>Induction of apoptosis in ovarian carcinoma cell lines</td>
<td>Increase in survival of murine intraperitoneal ovarian carcinoma</td>
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<td>Caspase-6</td>
<td>Expression vector hTERT-rev-caspase-6</td>
<td>Induction of apoptosis in hTERT-positive malignant glioma cells</td>
<td>Suppression of growth in subcutaneously established tumors in nude mice</td>
<td>[126]</td>
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<tr>
<td>7</td>
<td>Caspase-8</td>
<td>Adenoviral vector</td>
<td>Increased expression of X-ray-induced apoptosis in colon cancer DLD-1 cells</td>
<td>Increase in apoptosis in colon malignant DLD-1 cells after combination treatment with 5-Fu</td>
<td>—</td>
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<tr>
<td>8</td>
<td>Expression system hTERT–378/caspase-8</td>
<td>Adenoviral vector</td>
<td>Induction of apoptosis in human malignant glioma cells</td>
<td>Inhibition of growth in subcutaneously established U373-MG tumors in mice</td>
<td>[56]</td>
</tr>
<tr>
<td>9</td>
<td>Survivin</td>
<td>Expression system caspase-3/FKBP12 + CID</td>
<td>Significant induction of apoptosis in cancer colon DLD-1 cells</td>
<td>Intrapertioneal injection of CID induced apoptosis of endothelial cells expressing Caspase-3 and elimination of human microvessels engineered in immunodeficient mice</td>
<td>[80]</td>
</tr>
<tr>
<td>10</td>
<td>Caspase-9</td>
<td>Adenoviral vectors Ad-G/iCasp6 + Apaf-1</td>
<td>Augmentation of sensitivity of U373-MG glioma cells to etoposide-induced apoptosis</td>
<td>—</td>
<td>[109]</td>
</tr>
<tr>
<td>11</td>
<td>Caspase-9, APAF</td>
<td>Replication-deficient adenoviral vector pBabe/puro/Apaf-1 hTERT/FP2 expression construct</td>
<td>Enhancement of chemosensitivity in melanoma cell lines</td>
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<td>[113]</td>
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<tr>
<td>12</td>
<td>FADD</td>
<td>Heterozygous cell lines for TRAIL-R-mediated apoptosis of tumor cells of breast, cervical, prostate, lung, and colorectal cancer; enhancement of taxol-induced cell death</td>
<td>—</td>
<td>[127]</td>
<td></td>
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<tr>
<td>15</td>
<td>Survivin</td>
<td>Replication-deficient adenovirus Pad–T34A</td>
<td>Induction of apoptosis in cell lines of breast, cervical, prostate, lung, and colorectal cancer; enhancement of taxol-induced cell death</td>
<td>Suppression of de novo tumor formation, inhibition of the growth of established tumors and reduction on intraperitoneal tumor dissemination in breast cancer xenografts in immunodeficient mice</td>
<td>[73]</td>
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<tr>
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<td>Antisense digonucleotide</td>
<td>Induction of apoptosis in human mesothelioma H28 cells</td>
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<td>[138]</td>
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<tr>
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<td>Retroviral vector encoding a survivin-targeted ribozyme</td>
<td>Increase in apoptosis of human prostate cancer DU145 and PC-3 cells; increase in susceptibility to cisplatin-induced apoptosis</td>
<td>Prevention of tumor formation in androgen-independent prostate cancer xenografts in athymic nude mice</td>
<td>—</td>
<td>[87]</td>
</tr>
<tr>
<td>18</td>
<td>XIAP</td>
<td>Antisense digonucleotide G4 AS ODN</td>
<td>Induction of cell death in human NSCLC NIH–H660 cell lines; their sensitivity to the cytotoxic effects of daunobine, taxol, vinorelbine, and etoposide</td>
<td>Inhibition of H660 solid tumor growth in a xenograph model; in combination with vinorelbine significant delay in tumor growth</td>
<td>[40]</td>
</tr>
<tr>
<td>19</td>
<td>Anticaspase digonucleotide xiap AS PDON</td>
<td>Induction of apoptosis in multidrug-resistant bladder cancer T24 cells; escalation of doxorubicin-induced apoptosis</td>
<td>—</td>
<td>—</td>
<td>[6]</td>
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<td>20</td>
<td>FLIP sRNA</td>
<td>Sensitization of human tumor SV80 and KB cells for TRAIL–induced apoptosis</td>
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<tr>
<td>21</td>
<td>FLIP antisense oligodeoxyribonucleotide</td>
<td>Sensitization of human hepatocellular carcinoma cells to Fas–, TNF-R–, and TRAIL–mediated apoptosis</td>
<td>—</td>
<td>—</td>
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<tr>
<td>22</td>
<td>Smac</td>
<td>Recombinant adenovirus Ad CMV–Smac</td>
<td>Increase in apoptosis of ovarian carcinoma cells; sensitization of ovarian carcinoma cells to cisplatin and paclitaxel</td>
<td>—</td>
<td>[72]</td>
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<tr>
<td>23</td>
<td>pDNA3.1 vector containing full-length Smac CDNA</td>
<td>Sensitization of various tumor cells for apoptosis induced by death-receptor ligation or cytotoxic drugs</td>
<td>—</td>
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<tr>
<td>24</td>
<td>Cell-permeable peptides</td>
<td>Sensitization of various tumor cells for apoptosis induced by death-receptor ligation or cytotoxic drugs</td>
<td>Enhancement of the antitumor activity of TRAIL in an intracranial malignant glioma xenografts; sensitization of established tumors and survival of mice upon combined treatment with Smac peptides and TRAIL without detectable toxicity to normal brain tissue</td>
<td>—</td>
<td>[140]</td>
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<tr>
<td>25</td>
<td>Cell-permeable peptide SmacN7(R)8 sRNA</td>
<td>Selective reversion of the apoptosis resistance in human NSCLC NCI–H660 cells</td>
<td>Regression of tumor growth in combination with chemotherapy with little toxicity to the mice</td>
<td>—</td>
<td>[141]</td>
</tr>
</tbody>
</table>


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

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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.

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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, расцепляющие 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 — Apaf-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.