Caspases: pharmacological manipulation of cell death

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Caspases, a family of cysteine proteases, play a central role in apoptosis. During the last decade, major progress has been made to further understand caspase structure and function, providing a unique basis for drug design. This Review gives an overview of caspases and their classification, structure, and substrate specificity. We also describe the current knowledge of how interference with caspase signaling can be used to pharmacologically manipulate cell death.

Introduction

Apoptosis, or programmed cell death, is a common property of all multicellular organisms (1, 2). It can be triggered by a number of factors, including ultraviolet or γ-irradiation, growth factor withdrawal, chemotherapeutic drugs, or signaling by death receptors (DRs) (3, 4). The central role in the regulation and the execution of apoptotic cell death belongs to caspases (5–7). Caspases, a family of cysteinyl aspartate–specific proteases, are synthesized as zymogens with a prodomain of variable length followed by a large subunit (p20) and a small subunit (p10). The caspases are activated through proteolysis at specific asparagine residues that are located within the prodomain, the p20 and p10 subunits (8). This results in the generation of mature active caspases that consist of the heterotetramer $p20_2$–$p10_2$. Subsequently, active caspases specifically process various substrates that are implicated in apoptosis and inflammation. Their important function in these processes makes caspases potential targets for drug development. In this Review, we discuss the structures and functions of caspases as well as their role in novel approaches for treating cancer, autoimmune diseases, degenerative disorders, and stroke.

Structure of caspases

General overview. Caspases are zymogens (inactive enzyme precursors, which require a biochemical change to become an active enzyme) that consist of an N-terminal prodomain followed by a large subunit of about 20 kDa, p20, and a small subunit of about 10 kDa, p10 (Figure 1A) (5). In a number of procaspases, the p20 and p10 subunits are separated by a small linker sequence. Depending on the structure of the prodomain and their function, caspases are typically divided into 3 major groups (Figure 1A). The caspases with large prodomains are referred to as inflammatory caspases (group I) and initiator of apoptosis caspases (group II), while caspases with a short prodomain of 20–30 amino acids are named effector caspases (group III).

Caspase prodomains. The large prodomains of procaspases contain structural motifs that belong to the so-called death domain superfamily (9, 10). Death domains are 80- to 100-residue-long motifs involved in the transduction of the apoptotic signal. This superfamily consists of the death domain (DD), the death effector domain (DED), and the caspase recruitment domain (CARD) (11). Each of these motifs interacts with other proteins by homotypic interactions. All members of the death domain superfamily are characterized by similar structures that comprise 6 or 7 antiparallel amphipathic α-helices. Structural similarity suggests a common evolutionary origin for all recruitment domains (12). However, the nature of the homotypic interactions differs within the superfamily. DD and CARD contacts are based on electrostatic interactions, while DED contacts use hydrophobic interactions (13).

Procaspase-8 and -10 possess 2 tandem DEDs in their prodomain (14, 15). The CARD is found in procaspase-1, -2, -4, -5, -9, -11, and -12 (16, 17). DEDs and CARDs are responsible for the recruitment of initiator caspases into death- or inflammation-inducing signaling complexes, resulting in proteolytic autoactivation of caspases that subsequently initiates inflammation or apoptosis.

Structure of active caspase heterotetramers. Cleavage of a procaspase at the specific Asp-X bonds results in the formation of the mature caspase, which comprises the heterotetramer $p20_2$–$p10_2$ and causes release of the prodomain (Figure 1B). X-ray structures have been determined for mature caspase-1 (18, 19), caspase-2 (20), caspase-3 (21–23), caspase-7 (24–26), caspase-8 (27), and caspase-9 (28). The overall architecture of all caspases is similar. Each heterodimer (p10–p20) is formed by hydrophobic interactions resulting in the formation of several parallel β-sheets, composed of 6 antiparallel β-strands. Two heterodimers interact via a 12-stranded β-sheet that is surrounded by α-helices (Figure 1C). This so-called caspase fold is a unique quaternary structure among proteases and has been described only for caspases and for gingipain R, the cysteine protease from *Porphyromonas gingivalis* (29). In the caspase heterotetramer, the 2 heterodimers align in a head-to-tail configuration. Correspondingly, 2 active sites are positioned at opposite ends of the molecule. The architecture of the active center comprises amino acid residues from both subunits. The catalytic machinery involves a diad composed of a cysteine sulfohydryl group (Cys285) and a histidine imidazol ring (His237) (19). Both of them are located in the p20 subunit. The tetrahedral transition state of the cysteine protease is stabilized through hydrogen bonding with the backbone amide protons of Cys285 and Gly238. The asparagine of the substrate seems to be stabilized by 4 residues: Arg179 and Gln283 from the p20 subunit and Arg341 and Ser347 from the p10 subunit.

Substrate specificity and synthetic peptide inhibitors of caspases. Caspases are specific cysteine proteases, recognizing 4 amino acids, named S4-S3-S2-S1. The cleavage takes place typically after the

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Nonstandard abbreviations used: BIR, baculoviral IAP repeat; CARD, caspase recruitment domain; c-FLIP, cellular FLICE-inhibitory protein; DD, death domain; DED, death effector domain; DISC, death-inducing signaling complex; DR, death receptor; FLIP, FLICE-inhibitory protein; fmk, fluoromethyl ketone; IAP, inhibitor of apoptosis.

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Figure 1

Caspase structure. (**A**) The caspase family. Three major groups of caspases are presented. Group I: inflammatory caspases; group II: apoptosis initiator caspases; group III: apoptosis effector caspases. The CARD, the DED, and the large (p20) and small (p10) catalytic subunits are indicated. (**B**) Scheme of procaspase activation. Cleavage of the procaspase at the specific Asp-X bonds leads to the formation of the mature caspase, which comprises the heterotetramer $p20_2$–$p10_2$, and the release of the prodomain. The residues involved in the formation of the active center are shown. (**C**) The 3D structure of caspase-3 heterotetramer. Each heterodimer is formed by hydrophobic interactions resulting in the formation of mostly parallel β-sheets, composed of 6 antiparallel β-strands. Two heterodimers fit together with formation of a 12-stranded β-sheet that is sandwiched by α-helices. N and C termini of the small and large protease subunits are indicated.

C-terminal residue (S1), which is usually an asparagine (30). A list of substrate specificities of caspases is presented in Table 1. Interestingly, the preferred S3 position is an invariant glutamine for all mammalian caspases. Thus, specificity of caspase cleavage can be described as X-Glu-X-Asp. Caspase-1, -4, and -5 (group I; Figure 1) prefer the tetrapeptide sequence WEHD. Caspase-2, -3, and -7 have a preference for the substrate DEXD, whereas caspase-6, -8, and -9 prefer the sequence (L/V)EXD. Interestingly, the cleavage site between the large and small subunits for initiator caspases carries its own tetrapeptide recognition motif, which is remarkably consistent with the proposed mechanism of autoactivation of initiator caspases (31, 32).

Most of the synthetic peptide caspase inhibitors were developed based on the tetrapeptide caspase recognition motif. Therefore, the selectivity of inhibitors matches the caspase substrate specificities described above (Table 1). The introduction of an aldehyde group

Table 1
The substrate specificity of caspases

	Caspase	Substrate specificity
Group I	Caspase-1	WEHD
	Caspase-4	(W/L)EHD
	Caspase-5	(W/L)EHD
	Caspase-13	WEHD
	Caspase-14	WEHD
Group II	Caspase-2	DEHD
	Caspase-8	LETD
	Caspase-9	LEHD
	Caspase-10	LEXD
Group III	Caspase-3	DEVD
	Caspase-6	VEHD
	Caspase-7	DEVD

at the C terminus of the tetrapeptide results in the generation of reversible inhibitors (33), whereas a fluoromethyl ketone (fmk), a chloromethyl ketone (cmk) (34), or a diazomethyl ketone (dmk) (35) at this position irreversibly inactivates the enzyme.

Caspase signaling

Mechanisms of caspase activation. All caspases are produced in cells as catalytically inactive zymogens and must undergo proteolytic processing and activation during apoptosis. The effector caspases are activated by initiator or apical caspases. However, one central question of apoptosis is how initiator caspases are activated and how this activation is regulated to prevent spontaneous cell death. It is generally accepted that apical caspase activation takes place in large protein complexes that bring together several caspase zymogens (22–25, 36). All initiator caspases are characterized by the presence of a member of the DD superfamily (DED or CARD), which enables their recruitment into the initiation complex. Several activating complexes for initiator caspases have been reported so far.

The death-inducing signaling complex as an activating complex for procaspase-8 and -10. Procaspase-8 and -10 are apical caspases in apoptotic pathways triggered by engagement of death receptors. Several members of the TNF receptor (TNFR) superfamily (TNFR1, CD95 [Fas/APO-1], TRAIL-R1, TRAIL-R2, DR3, DR6) comprise DD in their intracellular domain and are, therefore, termed death receptors (3, 37). Triggering of CD95 and TRAIL-R1/R2 by corresponding ligands results in the formation of a death-inducing signaling complex (DISC) (38–42). CD95 and TRAIL-R1/R2 DISCs consist of oligomerized, probably trimerized, receptors, the DD-containing adaptor molecule FADD/MORT1 (Fas-associated death domain), 2 isoforms of procaspase-8 (procaspase-8/a [FLICE, MACHα1, Mch5] and procaspase-8/b [Machα2]), procaspase-10, and the cellular FLICE-inhibitory proteins (c-FLIP$_{L/S/R}$) (Figure 2A) (43, 44). The interactions between the molecules at the DISC are based on homotypic contacts. The DD of the receptor interacts with the DD of FADD, while the DED of FADD interacts with the N-terminal tandem DEDs of procaspase-8 and -10 and FLIP$_{L/S/R}$. Thus, DISC formation results in assembly of procaspase-8 and -10 molecules in close proximity to each other.

Activation of procaspase-8 is believed to follow an "induced proximity" model in which high local concentrations and favorable mutual orientation of procaspase-8 molecules at the DISC lead to their autoproteolytic activation (31, 45–47). There is strong evidence from a number of in vitro studies that autoproteolytic activation of procaspase-8 occurs upon oligomerization at the receptor complex (45–47). Furthermore, it has been demonstrated that dimers formed by procaspase-8 molecules possess proteolytic activity, and proteolytic processing of procaspase-8 occurs between precursor dimers (45). Interestingly, it has been shown that procaspase-8 and mature caspase-8 possess different substrate specificities (45). It is likely that conformational changes in the active center of caspase-8 occur upon processing to mature caspase-8.

The processing of procaspase-8/a/b at the DISC is depicted in detail in Figure 2. According to the 2-step model, the processing of procaspase-8 includes 2 cleavage events (39, 45). The first cleavage step occurs between the protease domains, and the second cleavage step takes place between the prodomain and the large protease subunit. Correspondingly, after the first cleavage step, p43/p41 and p10 subunits are formed (Figure 2, A and B). Both cleavage products remain bound to the DISC, p43/p41 by DED interactions and p10 by interactions with the large protease domain (48). As a result of the second cleavage step, p43/41 is processed to the prodomain p26/p24 and p18 (Figure 2C). Thus, the active caspase-8 heterotetramer is formed at the DISC. Subsequently, the mature caspase-8 heterotetramer is released to the cytosol to trigger apoptotic processes.

Procaspase-10 is also activated at the DISC, forming an active heterotetramer (15, 49). However, whether caspase-10 can trigger cell death in the absence of caspase-8 in response to CD95 or TRAIL-R1/R2 stimulation is controversial. Thus, the exact role of caspase-10 remains elusive.

The apoptosome as activating complex for procaspase-9. A number of apoptotic stimuli, such as cytotoxic stress, heat shock, oxidative stress, and DNA damage, lead to the release of cytochrome *c* from mitochondria. The release of cytochrome *c* is followed by the formation of a high–molecular mass cytoplasmic complex referred to as the apoptosome (50). In mammals the central scaffold protein of the apoptosome is a 140-kDa protein known as Apaf-1 (apoptotic protease–activating factor-1), which is a homologue of CED-4, a key protein involved in programmed cell death in the nematode *Caenorhabditis elegans* (51, 52). In the presence of cytochrome *c* and dATP, Apaf-1 oligomerizes to form a very large (700–1,400 kDa) apoptosome complex. Procaspase-9 is recruited to the complex by CARD interactions, which results in its activation (53). It has been biochemically demonstrated that activation of procaspase-9 occurs by dimerization (31). Moreover, it has been shown that proteolytic activation of procaspase-9 takes place upon dimerization and subsequent cleavage within an interdomain linker, which itself is important for stabilization of caspase-9 dimers.

The inflammosome as activating complex for caspase-1 and -5. The activation of the initiator caspase-1 and -5 takes place in a complex that was named the inflammosome (54). The inflammosome comprises procaspase-1 and -5 as well as the CARD-containing protein NALP-1. The formation of this complex results in the processing and activation of the cytokines IL-1β and IL-18, which play a central role in the immune response to microbial pathogens.

Effector caspase cascade. The activation of the effector caspase cascade differs between extrinsic (death receptor–mediated) and intrinsic (mitochondria-mediated) pathways.

In death receptor–mediated apoptosis, 2 types of signaling pathways have been established (55). So-called type I cells are characterized by high levels of DISC formation and increased amounts of active caspase-8 (Figure 3). Activated caspase-8 directly leads to the activation of downstream, effector caspase-3 and -7. In type II cells,

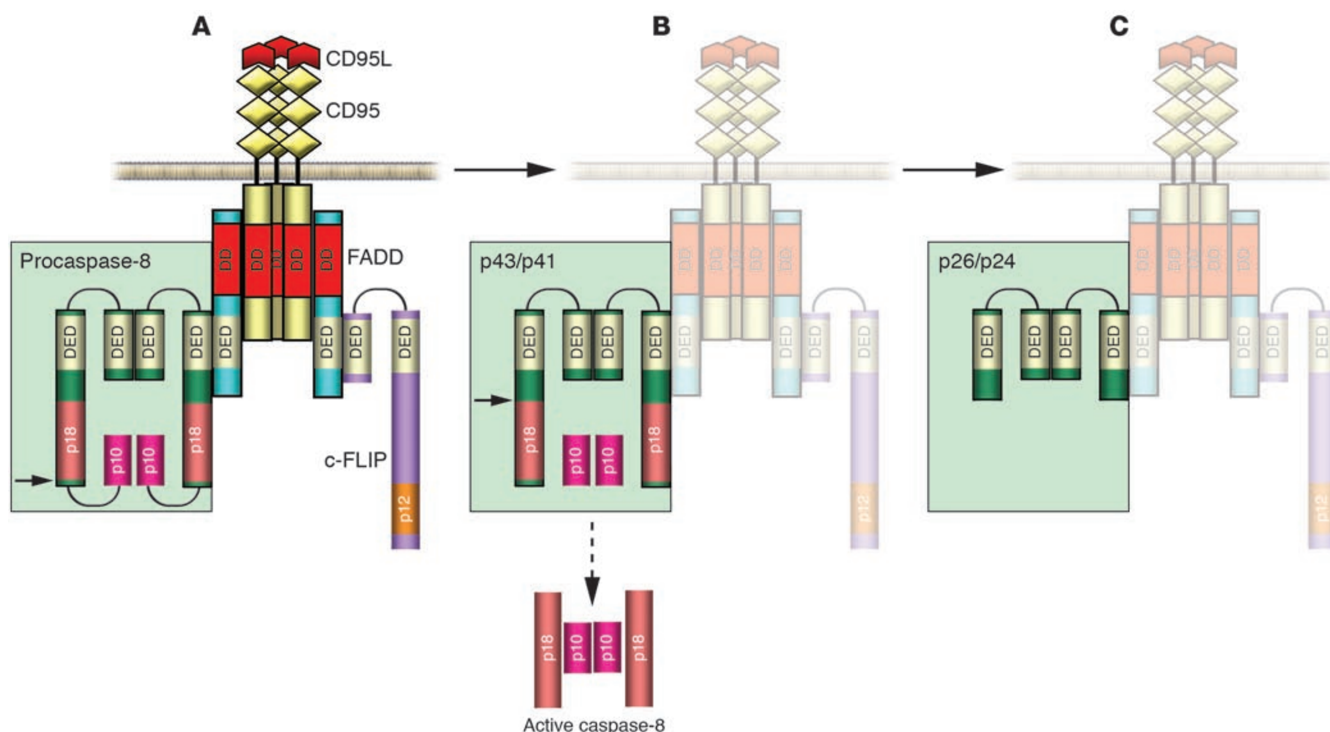

Figure 2
Scheme of procaspase-8 processing at the CD95 DISC. CD95 DISC formation is triggered by extracellular cross-linking with CD95L (depicted in red), which is followed by oligomerization of the receptor. FADD/MORT1 is recruited to the DISC by DD interactions (shown in red); procaspase-8 and -10 as well as c-FLIP proteins are recruited to the DISC by homophilic DED interactions (yellow). Upon recruitment to the DISC, procaspase-8 undergoes processing by forming dimers (depicted in green). (**A**) The first step of procaspase-8 cleavage occurs between 2 protease subunits. The site of cleavage is shown by a black arrow. As a result of the first cleavage step the p10 subunit is formed, which is not released into the cytosol but remains bound to the DISC as it is involved in the interactions with the large protease subunits. (**B**) The second cleavage step takes place between the prodomain and the large protease subunit at Asp216. As a result of this cleavage step the active caspase-8 heterotetramer is formed, which is then released into the cytosol. (**C**) Prodomain p26/p24 remains bound to the DISC.

there are lower levels of DISC formation and, thus, lower levels of active caspase-8. In this case, signaling requires an additional amplification loop that involves the cleavage of the Bcl-2–family protein Bid by caspase-8 to generate tBid and a subsequent tBid-mediated release of cytochrome *c* from mitochondria (56). The release of cytochrome *c* from mitochondria results in apoptosome formation, followed by the activation of procaspase-9, which in turn cleaves downstream, effector caspase-3 and -7. Type II signaling might be blocked by Bcl-2 family members such as Bcl-2 and Bcl-x_L.

In the intrinsic pathway, which is triggered by a number of factors, including UV or γ-irradiation, growth factor withdrawal, and chemotherapeutic drugs, the release of cytochrome *c* from mitochondria leads to apoptosome formation and activation of procaspase-9 (53). Subsequently, procaspase-9 cleaves effector caspase-3 and -7, which, correspondingly, initiate the death cascade. There are reports pointing toward a role of procaspase-2 in genotoxic stress acting upstream of mitochondria; however, this question requires further clarification (57, 58).

Cellular inhibitors of caspases. The action of caspases is regulated on several levels, including blockade of activation of caspases at the DISC as well as inhibition of enzymatic caspase activity (Figure 3). c-FLIP proteins are well-known inhibitors of death receptor–induced apoptosis (44, 59, 60). c-FLIPs possess 2 tandem DEDs at their N termini that facilitate their recruitment to the DISC. There are 3 c-FLIP isoforms described on the protein level, c-$FLIP_L$, c-$FLIP_S$, and c-$FLIP_R$ (61). Under conditions of overexpression, all isoforms inhibit activation of procaspase-8 at the DISC by blocking its processing (62) (Figure 3). At the same time, there is increasing evidence that c-$FLIP_L$, when present at the DISC at low concentrations, facilitates the cleavage of procaspase-8 at the DISC by forming c-$FLIP_L$–procaspase-8 heterodimers (45, 63).

The IAP (inhibitor of apoptosis) family of proteins includes 8 mammalian family members, including XIAP, c-IAP1, c-IAP2, and ML-IAP/livin (64–66). They specifically inhibit the initiator caspase-9 and the effector caspase-3 and -7 (Figure 3). The functional unit in IAP is the baculoviral IAP repeat (BIR), which contains about 80 amino acids folded around a central zinc atom. XIAP, c-IAP-1, and c-IAP2 contain 3 BIR domains each. The third BIR domain (BIR3) is involved in interactions with caspase-9 resulting in the inhibition of its activity (67). The linker region between BIR1 and BIR2 selectively targets caspase-3 and -7. The activity of IAPs is regulated by Smac/DIABLO, a structural homologue of the *Drosophila* proteins Reaper, Hid, and Grim (68, 69) (Figure 3). Smac/DIABLO is released from mitochondria and inhibits IAPs, which facilitates caspase activation during apoptosis. Omi/HtrA2 has been recently identified as another modulator of IAP function (70). Omi/HtrA2 is a mitochondria-located serine protease, which is released into the cytosol and inhibits IAPs by a mechanism similar to that of Smac.

IAPs are not the only natural inhibitors of caspases. The baculoviral p35 protein is a pan-caspase inhibitor, and it targets most

Figure 3
Caspase signaling and its modulation. In the extrinsic pathway, DISC formation leads to caspase-8 activation. Two signaling pathways downstream from the receptor were established. In type I cells (shown in light blue) caspase-8 directly cleaves caspase-3, which starts the death cascade. In type II cells (shown in light red) an additional amplification loop is required, which involves tBid-mediated cytochrome *c* release from mitochondria followed by apoptosome formation. Initiation of the intrinsic pathway results in mitochondria-mediated apoptosome formation, followed by caspase-9 and -3 activation, leading to destruction of the cell. Caspase action can be modulated on several levels. Activation of caspases at the DISC is inhibited by c-FLIP proteins; activation of effector caspases is inhibited by IAPs (see text). Effector caspases are shown in light green; cellular caspase inhibitors are presented in yellow. The targets for pharmacological modulation are shown with an orange arrow.

caspases, in contrast to IAPs, which affect only caspase-3, -7, and -9 (71). The mechanism of caspase inhibition by p35 involves the formation of an inhibitory complex that is characterized by a protected thioester link between the caspase and p35 (72). Structural analysis of the inhibitory complex between p35 and caspase-8 reveals a unique active-site conformation that protects the intermediary thioester link from hydrolysis. Another pan-caspase inhibitor, serpin CrmA, is derived from the cowpox virus (73). The mechanism

of CrmA inhibition is likely to involve covalent modification of the caspase active center.

Caspases as targets in drug development

Caspases, being the key effector molecules in apoptosis, are potential targets for pharmacological modulation of cell death (Figure 3). First, increased levels of caspase activity are often observed at sites of cellular damage in a number of diseases, including myocardial infarction, stroke, sepsis, and Alzheimer, Parkinson, and Huntington diseases. Inhibition of caspase activity for these diseases is predicted to be therapeutically beneficial. Second, discovery of drugs that selectively inhibit inflammatory caspases (caspase-1, -4, and -5) may help to control autoimmune diseases like rheumatoid arthritis. Finally, selective activation of caspases would be an approach in the treatment of cancer and chronic viral infections.

The approach of direct caspase inhibition is currently in the center of investigations. Proof-of-concept data have been obtained in several animal models, using suboptimal peptidyl inhibitors of caspases, such as zVAD-fmk, which shows substantial protection in rodent models of stroke, myocardial infarction, hepatic injury, sepsis, amyotrophic lateral sclerosis, and several other diseases (74–76). Furthermore, so-called small-molecule inhibitors of caspases, which have a nonpeptidyl origin, have been developed. One of these caspase inhibitors, IDN6556 (Pfizer), is already in phase II of clinical studies. It is a broad-spectrum caspase inhibitor that forms irreversible adducts with cysteine residues from the active site of caspases (77). IDN6556 is considered to be a candidate for the treatment of acute–tissue injury diseases, which are characterized by excessive apoptosis. This inhibitor is currently being tested in treatment of liver diseases, including HCV and ischemia/reperfusion injury in liver transplantation.

Among the inhibitors of inflammatory caspases, VX-740 (Vertex Pharmaceuticals Inc.) is also in phase II of clinical studies (78). It is a selective and reversible inhibitor of caspase-1 that is developed for treatment of rheumatoid arthritis.

Another promising strategy involves selective activation of caspases in cancer cells, leading to induction of apoptosis. An important contribution to this strategy was achieved by the approach of "forward chemical genetics" (79). This involves screening of small molecules for their ability to perturb cellular pathways and subsequently identifying the specific targets of the active compounds. A number of potential drugs for selective induction of apoptosis were found by high-throughput screening of the compounds activating caspase-3 as a central suicide caspase. Among them are a small molecule, PETCM [α-(trichloromethyl)-4-pyridine ethanol] (80); carbamate and indolone classes of compounds (81); and MX2167, MX116407, MX128504, and MX90745 (82). These compounds engage different pathways; e.g., PETCM accelerates apoptosome formation by interacting with the inhibitor prothymosin-α (80); carbamate and indolone classes of compounds promote Apaf-1 oligomerization and, thereby, apoptosome formation with the subsequent activation of caspase-3 and -9 (81); the action of MX2167 is mediated via the transferrin receptor that is highly overexpressed in a number of tumors; MX116407 is a vascular targeting agent; and MX128504 targets a novel cytoplasmic protein affecting IGF growth signaling pathways. At the same time, all these drugs are characterized by selective induction of apoptosis, and some of them are in preclinical and phase I studies.

Strategies to selectively target cancer cells also involve the application of antibodies coupled to active caspases. There were reports on a chimeric protein, referred to as immunocasp-3, that comprises a single-chain anti–erbB2/HER2 antibody, a translocation domain of *Pseudomonas* exotoxin A, and constitutively active caspase-3 (83). Immunocasp-3 was shown to selectively bind to erbB2/HER2–overexpressing cancer cells, followed by its internalization and lysosomal cleavage. As a result a COOH-terminal caspase-3–containing fragment is released, which translocates to the cytosol and induces apoptosis. Following this study there was a report on erbB2/HER2–targeted immunocasp-6, which can directly cleave lamin A, leading to nuclear damage, and induce programmed cell death (84). These studies provide the platform for the development of novel therapeutic protocols against tumors that overexpress erbB2/HER2.

In addition to direct targeting of caspases, other strategies involve modulation of cellular caspase inhibitors, such as IAPs, c-FLIPs, and Smac (Figure 3). Some cancers are characterized by overexpression of IAPs that are associated with resistance to apoptosis. The prototypic example is melanoma IAP (MILAP, also known as livin/KIAP), which is found at high levels in melanomas (66). Therefore, strategies of downregulating IAPs play an important role, as this would result in caspase activation and subsequent apoptosis induction in cancer cells. XIAP antisense molecules can directly induce apoptosis as well as sensitize cells to chemotherapy and irradiation (85). Antisense XIAP oligonucleotides are currently in phase I clinical studies.

Another approach to target IAPs is the identification of small molecules that bind to IAPs and prevent their inhibition of caspases. There are 3 main screening strategies to search for IAP antagonists. First, peptides that structurally resemble the N terminus of Smac were shown to bind to the BIR3 pocket of XIAP, subsequently leading to inhibition of XIAP (86). The development of these peptides is promising; however, further work is required to determine whether these peptides can induce apoptosis or sensitize cells to chemotherapy. In a second strategy, phage display was used to identify XIAP-binding peptides. This screen identified peptides unrelated to Smac that bind to the BIR2 domain and can directly induce cell death in leukemia cells (87). In a third approach, biochemically based assays were used to identify small molecules and peptides that inhibit XIAP. The screening was based on the reversed XIAP-mediated repression of caspase-3. Through these screens, both peptidyl and nonpeptidyl XIAP inhibitors were identified (85, 88). The small molecules identified by Wu and colleagues appear to be capable of sensitizing TRAIL-resistant cells toward TRAIL-induced apoptosis.

In addition to targeting of IAPs in cancer, IAPs might be used in gene therapy strategies to reduce neuronal cell death that is followed by stroke or brain injury. Virus-mediated delivery of IAP into the brain preserved neurons in rodent models of stroke and other types of experimental injury (89). These data indicate that modulation of IAP action might be also used in treating neurological diseases.

Given the current status of research on caspases as targets for pharmacological manipulation, the value of certain strategies is variable. Notably, despite the attraction of using caspase inhibitors, this approach might be ineffective. Blocking caspases often results in triggering of caspase-independent cell death accompanied by the release of cytochrome *c*, AIF, and EndoG from mitochondria. Thus, how to prevent cross-talk between different pathways involved is not really clear. Strategies of selective activation of caspases in cancer cells by various drugs or site-directed delivery, however, may be more promising and might find their way into the clinic. Moreover,

certain compounds might be applied in combination with standard chemotherapy and are likely to be more efficient. Finally, targeting of IAPs by, e.g., IAP antagonists appears to be a potential clinical application and may become a tool in cancer therapy.

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