Apoptosis-Modulating Drugs for Improved Cancer Therapy

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Key Words

Apoptosis • Autophagy • Endoplasmic reticulum stress •
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Abstract

Resistance to cell death induction has been recognized as a hallmark of cancer. Increasing understanding of the underlying molecular events regulating different cell death mechanisms like apoptosis, endoplasmic reticulum stress, autophagy, necroptosis and others has opened new possibilities for targeted interference with these pathways. While conventional chemotherapeutic agents usually inhibit cell cycle progression, DNA replication or mitosis execution, novel agents like small molecule kinase inhibitors also target survival-related kinases and signaling pathways and contribute to overcome resistance to chemotherapy and apoptosis. Additionally, antibodies targeting cellular death receptors have been described to specifically target tumor cells only. This review briefly highlights the pathways involved in (apoptotic) cell death and summarizes the current state of development of specific modulators of cell death and how they can help to improve the tolerability of chemotherapy regimens and increase survival rates in patients with advanced cancer diseases.

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Introduction

Since its first recognition as a physiologic process of cellular turnover and tissue homeostasis 4 decades ago [1], programmed cell death mechanisms have received increasing attention as central regulators of (patho-)physiologic conditions. The uncontrolled initiation and execution of various cell death programs have been linked to various disease conditions, e.g. transplant rejection, autoimmune diseases or ischemic heart failure. Especially the ability to resist cell death has been identified as a key feature of malignant cells and has therefore been considered a hallmark of cancer [2].

Conventional chemotherapeutic agents like 5-fluorouracil, irinotecan, doxorubicin or platinum derivatives unspecifically interfere with DNA and RNA metabolism and replication during the S phase of the cell cycle, while agents like taxol derivatives arrest cells during mitosis and lead to mitotic catastrophe. Yet, these commonly used and well-established compounds lead to cell death induction only secondarily and show a high rate of resistance development [3, 4]. The introduction of targeted therapies using small molecule kinase inhibitors like sorafenib, everolimus, imatinib or growth factor-related antibodies like cetuximab or bevacizumab has shown

that a direct interference with survival and cell death-related signaling pathways can significantly improve patient survival for various cancers [5–9]. Recently, the increasing knowledge about the molecular mechanisms involved in cell death initiation, execution and regulation has opened new possibilities to directly target the cellular cell death machinery in a more specific manner.

We will here briefly review the current concepts of cell death mechanisms in general and of cancer-related alterations with a special focus on novel targeted agents for modern personalized cancer chemotherapy.

Types of Cell Death: Apoptosis, Necroptosis and Autophagy

Although there is increasing evidence that the three major ways of cell death, apoptosis, necrosis/necroptosis and autophagy, share overlapping molecular features and pathways and can occur also in parallel under the same conditions [10–13], they will be discussed separately here. Mitotic catastrophe represents a fourth way of cell death but will not be discussed here, and we refer to other excellent reviews on this topic [14, 15].

Apoptosis

Apoptosis is considered the classical way of programmed cell death. Morphologically, typical features comprise the condensation and pyknosis of nuclei, the formation of membrane blebbings and the complete consumption of apoptotic cells by phagocytes without an inflammatory response [16–18].

The apoptotic machinery can be activated by various intrinsic and extrinsic stimuli (fig. 1). The extrinsic pathway is activated by binding of specific ligands to so-called death receptors of the tumor necrosis factor (TNF) receptor superfamily at the cellular surface [19, 20]. Ligand binding to its cognate receptor (FasL-Fas/CD95, TRAIL-DR4 or DR5, TNF-TNFR1) leads to receptor trimerization and activation of intracellular death domains and recruitment of death domain-containing adapter proteins like Fas-associated death domain or TNF receptor-associated death domain that form a death-induced signaling complex which contains the proform of the initiator caspases 8 or 10 [17, 21, 22]. Extrinsic apoptosis induction is controlled at the level of signal transduction by inhibitory proteins like cellular FLICE-inhibitory protein [23] and at the level of ligand binding by the expression of decoy receptors (DcR1, DcR2 and DcR3) lacking intracellular death domains [24].

Various factors can activate the intrinsic or mitochondria-mediated apoptosis cascade. Among them are hypoxia or ischemia (antiangiogenic therapy), reactive oxygen species production (radiation therapy) or inhibition of growth factor signaling (small molecule kinase inhibitors or antibody therapy) [17]. These intracellular stress signals activate proapoptotic members of the Bcl-2 (B cell lymphoma 2) family [25, 26], e.g. the BH3-only proteins Bad or Bim which in turn inhibit the function of anti-apoptotic members of this protein family like Bcl-2, Bcl-xL or Puma/Noxa. This allows Bax and Bak, other proapoptotic members of the Bcl-2 family, to dimerize and translocate to the outer mitochondrial membrane and to trigger the release of proapoptotic mediators like cytochrome c, Ca^{2+} or the inhibitors of apoptosis proteins (IAPs) Smac/DIABLO into the cytosol [27–30]. Cytochrome c then forms a complex with the apoptotic protease-activating factor 1 to build the so-called apoptosome which attracts and cleaves procaspase 9 to activate the caspase cascade [31–33].

Recent findings demonstrate that the Bcl-2 family also affects the integrity of other intracellular membranes and compartments like the endoplasmic reticulum (ER) leading to the release of Ca^{2+} which activates several intracellular cytotoxicity mechanisms like endonucleases, transglutaminase or proteases [34–38]. Additional factors inducing ER stress-mediated apoptosis include oxidative or chemical damage of the ER membrane and the activation of the unfolded protein response (UPR) [39]. Briefly, misfolded proteins activate signaling pathways like the transcription factor ATF6, which mediates the transcription of UPR target genes including chaperones of the heat shock protein 70 and 90 families (e.g. BiP or GRP94) [40, 41]. Alternatively, ER stress can activate transmembrane proteins located in the ER membrane like IRE1 and PERK [39]. These molecules inhibit general protein synthesis and activate the noncanonical apoptosis response via activation of the stress kinase JNK and subsequently of caspase 12 [42–45].

The execution of apoptotic cell death is mediated by a family of cysteinyl aspartate proteinases (caspases) consisting of at least 14 members. Caspases are synthesized as inactive proforms consisting of a large and a small subunit that upon cleavage of the propeptide form the active enzyme complex [46]. Caspases show a hierarchical organization with several initiator caspases (see below) and the effector caspases 3, 6 and 7 that mediate the typical cleavage of cellular structures during apoptosis execution [47]. Active caspases induce the proteolytic cleavage of numerous cellular proteins [17, 48], e.g. apoptosis regula-

Fig. 1. Schematic representation of cell death pathways. See main text for details.

tors like other caspases or Bcl-2 family members [49, 50], structural proteins like cytokeratins [51, 52] or ROCK-1 kinase [53], cell cycle regulators like Rb [54] or p21^{cip1/waf1} [55] or DNA-metabolizing enzymes like the caspase-activated DNase inhibitor [56, 57]. Caspase activity itself is controlled by the IAP family of BIR-domain containing proteins like XIAP, cIAP-1, cIAP-2 or survivin [58–60]. Interestingly, XIAP and cIAP-1 themselves are target molecules for caspases and must be cleaved to execute apoptosis [61]. IAPs also control the death-receptor-mediated induction of necroptosis by inhibiting receptor-interacting proteins (RIPs) associated with necroptotic cell death [62].

Necrosis and Necroptosis

Necrotic cell death has been characterized as a morphologic distinct form of cellular demise leading to swelling and rupture of organelles and cells due to lack of ATP and breakdown of ion gradients. Unlike apoptosis, this causes an enhanced inflammatory response in affected tissues [17]. Recent data also show a physiologic role for necrosis, e.g. during intestinal cell renewal, and further data also evidence a programmed control and execution of this form of cell death [63–66], which is therefore now called necroptosis [67]. Defects in death receptor signaling and inhibition of caspase 8 activity stabilize the receptor-interacting proteins RIP1 and its homologue RIP3 and activating their kinase domains. RIP1 can translo-

cate to the mitochondria and rapidly disrupt the ADP/ATP translocase complex which leads to the typical loss of ATP [68]. Although the exact mechanism of RIP1 activity is still unclear, production of reactive oxygen species, ceramide and interference with stress-related JNK have been proposed and thus provide a crosslink to apoptotic and autophagic cell death pathways, too (fig. 1) [66, 69–70].

Autophagy

Autophagy has originally been described as a physiologic response to nutrient deprivation and means of cellular survival under stress conditions. Morphologically, autophagy is characterized by the formation of cytoplasmic vacuoles containing cellular organelles or protein aggregates and the absence of typical features of apoptotic cell death. Biochemically, cell death is executed without activation of caspases. Formation of autophagic vacuoles involves several members of the ATG gene family that can recruit the BH3-domain-containing regulator beclin 1 [71] and the phosphoinositide-3-kinase (PI3K) member VPS34. This complex then activates other ATG members that recruit the protein LC3 and conjugate it with phosphatidylethanolamine that finally forms the autophagosome vesicle. The mature autophagosome then fuses with a lysosome to an autophagolysosome leading to the digestion of its content [72, 73].

Because of its role in protein turnover and recycling of cellular components, autophagy is also closely linked to ER stress and UPR-induced apoptotic cell death [74]. Yet, it is still under debate, whether autophagy may be a mere cell survival mechanism either antagonizing apoptosis pathways or contributing and enhancing cell death programs under certain conditions like failure of caspase activation or defects in Bax or Bak functions [75–77].

Cell Death Deficiency in Cancer

Defects in initiating or executing cell death programs are considered a hallmark of malignant cells [2]. This deficiency is responsible for resistance to conventional and targeted chemo- and radiotherapy as well as the limitations of overall survival in cancer patients and the development of metastasis [78]. As cell death deficiency provides a significant advantage for tumor cells, all of the pathways described above can be altered in cancer. Overexpression of antiapoptotic molecules like Bcl-2, Bcl-xL, IAPs or survivin are therefore commonly detected in human cancer and linked to poor prognosis

[79–86]. Consequently, also the loss of proapoptotic factors like death receptors, adapter molecules or caspases has been observed in this context [87–93]. Interestingly, also the commonly observed increased expression or activity of growth-factor signaling cascades (e.g. epidermal or vascular endothelial growth factors) can influence cell death sensitivity by their convergence with mitogen-activated protein kinase or PI3K/Akt signaling. Active Akt provides strong survival signals by phosphorylating and thus inactivating e.g. proapoptotic molecules like Bad or caspase 9 or forkhead transcription factors regulating the expression of e.g. Fas or Bim [94–96]. Downstream, Akt also activates the mammalian target of rapamycin complex, which is considered a master regulator of hypoxia response and autophagy induction [11, 12, 97–99].

Novel Compounds Enhancing Cell Death Responses

Although most chemotherapeutic agents used today can at least partially induce apoptotic cell death, more specific drugs targeting the pathways described above have recently been developed. As most of the compounds described below are in early clinical trials, no concluding information about the overall efficacy in terms of prolongation of survival time and response rates is so far available.

Death Receptor Pathway

While the stimulation of the Fas/CD95 system is limited by severe systemic toxicities including acute liver failure, the DR4/DR5 and TNF apoptosis-inducing ligand (TRAIL) system represent promising targets in early phase clinical trials.

Several agonistic and fully human monoclonal antibodies to DR5 have recently been introduced [100]. Conatumab (AMG 655) [101], tigatuzumab (CS-1008) [102], lexatumumab [103] and drozitumab (PRO95780) [104] have been investigated in several phase 1 and phase 2 trials as single agents or in combination with various chemotherapeutic regimens for different solid tumors [105–109]. Mapatumumab represents the only available fully human monoclonal antibody activating DR4 [110–114].

Dulanermin is a recombinant human fragment consisting of 167 amino acids of the natural Apo2L/TRAIL ligand and mediated proapoptotic functions by binding to DR4 and DR5 [115]. So far, dulanermin has been investigated in phase 1 and phase 2 trials for advanced solid

tumors including colorectal and non-small-cell lung cancer revealing a good safety profile [116, 117].

Besides these proapoptotic receptor agonists, other strategies have recently been proposed to enhance the effect of receptor-mediated apoptosis induction. Interference with protein turnover or receptor trafficking, e.g. by application of proteasome inhibitors like bortezomib or protein deacetylase inhibitors, has been shown to increase either death receptor availability or to decrease the availability and function of inhibitory molecules like cellular FLICE-inhibitory protein [118–122].

Mitochondrial Pathway

To activate the mitochondria-driven apoptosis, inhibitors of Bcl-2 function and BH3 mimetics have been designed [123, 124]. One of the first approaches was the antisense-based inhibition of Bcl-2 expression using the DNA antisense oligonucleotide oblimersen (G3139, genasense) [125]. In several clinical trials this strategy proved to lower the apoptotic thresholds in various models and increased the sensitivity towards other chemotherapeutic agents [126–130]. Although results from these trials are promising, approval of this drug is still pending, and additional effects like stimulating Toll-like receptors by CpG motifs contained in the oblimersen sequence are discussed but not fully understood [131]. Recently, the use of short interfering RNA molecules (siRNAs) has been shown to be more powerful in suppressing the expression of target genes. Consequently, several approaches have been made to target antiapoptotic molecules like Bcl-2 with this technique [132]. Although preclinical findings demonstrated a good efficacy in silencing Bcl-2 and thus shifting the apoptotic threshold [133, 134], the clinical applicability of siRNA-based therapies is still limited due to unsolved pharmacological problems such as drug delivery or drug targeting issues and the low in vivo stability of siRNA molecules.

Several small molecule inhibitors targeting the antiapoptotic Bcl-2 family members have been investigated recently. These so-called BH3 mimetics directly interfere with the protein structure of Bcl-2, Bcl-xL, Bcl-w or Mcl-1 and thus inhibit the antiapoptotic function of these molecules [124]. Among them, the preclinically most intensively studied compound obatoclax (GX15-070) [135–137] and gossypol (AT-101) [138–140] showed very promising results and are currently undergoing clinical testing. ABT-737 and its orally available derivative navitoclax (ABT-263) bind to Bcl-2, Bcl-xL and Bcl-w and sequester proapoptotic BH3 domain proteins, which promotes the oligomerization of proapoptotic Bax and

Bak [141]. Navitoclax showed a good safety profile in phase 1 trials [142, 143] and demonstrated synergistic effects with other chemotherapeutics in various cancer models [144–146].

ER Stressors and UPR Inducers

Although ER stress and the UPR are themselves potent inducers of noncanonical apoptosis signaling, no specific agents activating this pathway are yet available. Several established compounds interfering with protein metabolism and turnover can induce UPR activation. Here, proteasome inhibitors like bortezomib [147], heat shock protein inhibitors like geldanamycin (17-AAG) [148] or protein deacetylase inhibitors like panobinostat [149–151] can rapidly induce ER stress-mediated apoptosis in cancer cells.

Modulators of Autophagy

As discussed, it is still under debate whether autophagy acts as a defense mechanism against chemotherapy or if it contributes to the execution of cell death programs. Interestingly, several well-established chemotherapeutic agents have recently been demonstrated to interfere with autophagy programs in tumor cells [152]. While inhibitors of survival pathways blocking the PI3K/Akt/mammalian target of rapamycin axis (e.g. BEZ235, AZD8055 or rapamycin analogs) activate the autophagosome formation, maturation and degradation of autophagolysosomes is inhibited by chloroquine and hydroxychloroquine [153]. Depending on the cellular context, both pathways can contribute to cell death in cancer cells. The latter compounds have been investigated in various trials to overcome autophagy-mediated survival and resistance [154–156].

Inhibitors of IAPs and Survivin

Caspase activity is tightly controlled by IAPs. Mimetics of Smac/DIABLO have demonstrated high efficacy in inhibiting IAP function and sensitize cancer cells to cytotoxic agents [157]. Yet, none of the currently available peptidic or nonpeptidic Smac mimetics has been investigated in clinical trials. Antisense oligonucleotides against the IAP inducer survivin (LY2181308) or against XIAP (AEG35156) have also shown good tolerability in phase 1 trials [158–160] but need to be confirmed in larger patient cohorts [161].

Conclusion

The growing understanding of the complexity of cell death programs has opened new possibilities to exploit these pathways for targeted cancer therapies. Although most of the described compounds and agents are still in preclinical or early clinical evaluation and evidence-based data on efficacy and potency are not yet available, the prospect of combining specific apoptosis modulators or inducers with established chemotherapeutic agents will dramatically change our view on cancer chemotherapy. As these modulators span the whole range from monoclonal antibodies targeting death receptors to mod-

ulators of intracellular signaling cascades or protein turnover, multiple new studies and study designs need to be conducted and validated to help improving patient care and overall survival of cancer patients. Four decades after the declaration of war on cancer, the war is still not won, but these new weapons can help turn the page for the benefit of numerous patients.

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