Signaling through death receptors in cancer therapy
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Apoptosis — the cell’s intrinsic program for death — plays a central role in regulation of tissue homeostasis. Accordingly, tipping the balance between cell death and proliferation in favor of cell survival can result in tumor formation. Also, killing of cancer cells by cytotoxic therapies (e.g. chemotherapy, γ-irradiation, immunotherapy or suicide gene therapy) largely depends on intact apoptosis programs in cancer cells. To this end, it is implied that death receptor signaling contributes to the efficacy of cancer therapy. Failure to undergo apoptosis in response to anticancer therapy can therefore result in resistance. Thus, insights into the mechanisms regulating apoptosis in response to anticancer therapy and the ways in which cancer cells evade apoptosis might provide new opportunities for drug development.

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Abbreviations
DISC death-inducing signaling complex
FADD Fas-associated death domain
FLIP FLICE-inhibitory protein
IAP inhibitor-of-apoptosis protein
IFN interferon
PED/PEA-15 phosphoprotein enriched in diabetes/phosphoprotein enriched in astrocytes-15kDa
PPAR peroxisome proliferator-activated receptor
Smac second mitochondria-derived activator of caspase
TNF tumor necrosis factor
TRAIL TNF-related apoptosis-inducing ligand

Introduction
Killing of tumor cells by cytotoxic therapies (e.g. chemotherapy, γ-irradiation, immunotherapy or suicide gene therapy) is predominantly mediated by triggering apoptosis in cancer cells [1]. Apoptosis or programmed cell death is a distinct intrinsic cell death program that occurs in various physiological and pathological situations [2]. Apoptosis pathways are tightly controlled and anti-apoptotic mechanisms have been implicated in tumor cell resistance [3]. However, the concept that apoptosis represents the major mechanism by which cancer cells are eliminated does not universally apply, and caspase-independent apoptosis or other modes of cell death have also to be considered as cell death responses to cytotoxic therapy [4]. Thus, a better understanding of these diverse modes of cell death in cancer therapy will provide a molecular basis for new strategies targeting death pathways in resistant forms of cancer.

Apoptosis signaling pathways
Anticancer drug treatment usually results in activation of caspases, a family of cysteine proteases that act as common death effector molecules in various forms of cell death [5]. Caspase activation can be initiated in two different ways: at the plasma membrane upon ligation of death receptors (the receptor pathway) or at the mitochondria (the mitochondrial pathway) [6]. Stimulation of death receptors of the tumor necrosis factor (TNF) receptor superfamily, such as CD95 (APO-1/Fas) or the TNF-related apoptosis-inducing ligand (TRAIL) receptors, results in activation of the initiator caspase-8, which can propagate the apoptosis signal by direct cleavage of downstream effector caspases such as caspase-3 [7]. The mitochondrial pathway is initiated by the release of apoptogenic factors such as cytochrome c, apoptosis-inducing factor, second mitochondria-derived activator of caspase (Smac/Diablo, Omi/HtrA2, endonuclease G, caspase-2 or caspase-9 from the mitochondrial intermembrane space [8]. The release of cytochrome c into the cytosol triggers caspase-3 activation through formation of the cytochrome c/Apaf-1/caspase-9-containing apoptosis complex, whereas Smac/Diablo and Omi/HtrA2 promote caspase activation through neutralizing the inhibitory effects of inhibitor-of-apoptosis proteins (IAPs) [8]. Links between the receptor and the mitochondrial pathway exist at different levels. Upon death receptor triggering, activation of caspase-8 results in cleavage of Bid, a Bcl-2 family protein with a BH3 domain only, which in turn translocates to mitochondria to release cytochrome c, thereby initiating a mitochondrial amplification loop [8]. In addition, cleavage of caspase-6 downstream of mitochondria can feed back to the receptor pathway by cleaving caspase-8 [9].

The death receptor family
Death receptors and their ligands
Death receptors belong to the TNF/nerve growth factor superfamily of cell surface receptors, and contain a cytoplasmic protein motif termed the death domain that enables death receptors to engage the cell’s apoptotic machinery [7]. Currently, six different death receptors...
have been identified, including CD95 (APO-1/Fas), TNFRI, DR3 (TRAMP), TRAIL-R1 (DR4), TRAIL-R2 (DR5) and DR6. Death receptors are activated upon binding to their corresponding ligands or by agonistic antibodies. Death receptor ligands of the TNF superfamily comprise CD95 ligand, TNFz, lymphotixin-z (this and TNFz bind to TNFRI), TRAIL and TWEAK (a ligand for DR3).

Signalizing through death receptors
Ligation of death receptors, such as CD95 or the agonistic TRAIL receptors TRAIL-R1 and TRAIL-R2, by their cognate ligands or agonistic antibodies results in receptor trimerization, clustering of the receptors’ death domains and recruitment of adaptor molecules (e.g. Fas-associated death domain [FADD]) through homophilic interaction mediated by the death domain [10,11]. FADD in turn recruits caspase-8 to the activated CD95 receptor to form the CD95 death-inducing signaling complex (DISC). Oligomerization of caspase-8 upon DISC formation drives its activation through self-cleavage. Caspase-8 then activates downstream effector caspases such as caspase-3. Caspases cleave several different substrates in the cytoplasm or nucleus leading to many of the morphological features of apoptotic cell death [12]. Besides caspase-8, caspase-10 is also recruited to the TRAIL DISC [13]. However, the importance of caspase-10 for apoptosis induction is controversial [13,14].

Death receptors and cancer therapy
CD95 and cancer therapy
The CD95 receptor/ligand system is a key signaling pathway involved in the regulation of apoptosis in several different cell types (e.g. in the immune system) [15,16]. CD95, a 48 kDa type I transmembrane receptor, is expressed on activated lymphocytes, in a variety of tissues of lymphoid or non-lymphoid origin, as well as on tumor cells [15]. CD95 ligand, a 40 kDa type II transmembrane molecule, occurs in both a membrane-bounded and a soluble form [15].

The CD95 system has also been implicated in chemotherapy-induced tumor cell death in several studies [17–22]. Expression of CD95 was found to be upregulated upon treatment with anticancer drugs, which in turn triggered the CD95 pathway in an autocrine or paracrine manner. In support of this concept, an increase of CD95 ligand mRNA and protein expression was found in a variety of different tumor cell lines in vitro and also ex vivo in tumor cells derived from primary patients. Moreover, soluble antagonistic CD95 receptors, antagonistic CD95 ligand antibodies or dominant-negative FADD reduced drug-induced apoptosis under certain circumstances. Furthermore, resistance to CD95-triggered apoptosis has been associated with cross-resistance to various anticancer agents in some leukemia and solid tumor cell lines. This indicates that cell death signaling upon physiological stimuli such as CD95 triggering and chemotherapy require, at least in part, similar pathways (e.g. the CD95 system).

Despite the reproducibility of these findings in a variety of different model systems, other reports challenged the concept that CD95 signaling is involved in drug-mediated cell death [23–25]. Antagonistic antibodies against CD95 ligand or CD95 did not confer protection against apoptosis induced by cytotoxic drugs in other cell lines. The discrepancies in findings might be related to, firstly, the relative contribution of the death receptor versus the mitochondrial pathway to drug-induced apoptosis, depending upon the anticancer agent used, dose and kinetics; secondly, to cell-type-specific differences; or thirdly, to differences in blocking reagents used to inhibit the CD95/CD95 ligand interaction. Despite the involvement of the CD95 system in anticancer drug-induced apoptosis under certain circumstances, most cytotoxic drugs are considered to primarily initiate cell death by triggering a cytochrome c/Apaf-1/caspase-9-dependent pathway linked to mitochondria [26].

TRAIL and cancer therapy
TRAIL is constitutively expressed in many tissues, as are the agonistic TRAIL receptors TRAIL-R1 and TRAIL-R2 [27]. TRAIL-R3, -R4 and -R5 are antagonistic decoy receptors that bind TRAIL but do not transmit a death signal [27]. The TRAIL ligand and its signaling pathway are of special interest for cancer therapy because TRAIL has been shown to predominantly kill cancer cells, while sparing normal cells [27]. The underlying mechanisms for this differential sensitivity of malignant versus non-malignant cells have not been clearly defined. One possible mechanism of protection of normal tissues is thought to involve a set of antagonistic decoy receptors, which compete with TRAIL-R1 and TRAIL-R2 for binding to TRAIL.

Recombinant soluble TRAIL induced apoptosis in a broad spectrum of cancer cell lines, including colon, breast, lung, pancreas, prostate, renal and thyroid carcinomas, malignant brain tumors, Ewing tumor, osteosarcoma, neuroblastoma, leukemia and lymphoma [27,28]. TRAIL also exhibited potent tumoricidal activity in vivo in several xenograft models, such as colon carcinoma, breast carcinoma, malignant glioma and multiple myeloma [27,28]. Furthermore, monoclonal antibodies that engage the TRAIL receptors TRAIL-R1 or TRAIL-R2 also demonstrated potent antitumor activity both against tumor cell lines and in preclinical cancer models [27,28]. In addition to recombinant soluble TRAIL ligand, several gene therapy approaches have been developed to specifically target tumor cells. To this end, an adenoviral vector expressing the TRAIL gene from the hTERT promoter elicited high levels of TRAIL expression and apoptosis specifically in breast cancer cells, whereas only minimal
transgene expression and toxicity was detected in normal human primary mammary epithelial cells [29]. Intrale- sional administration of adenoviral TRAIL effectively suppressed the growth of human breast cancer xenografts, resulting in long-term tumor-free survival of mice [29]. The antitumor effect of an intratumoral administration of TRAIL expressed by an adenoviral vector in an in vivo model of breast carcinoma was attributed to direct tumor cell killing, as well as to a bystander effect through presentation of TRAIL by transduced normal cells [30]. Importantly, recent evidence suggests that, besides triggering apoptosis, TRAIL is able to induce survival and proliferation in cancer cells resistant to TRAIL-induced apoptosis, which is mediated by the transcription factor nuclear factor-κB [31]. Thus, the death-inducing ligand TRAIL might paradoxically promote tumor growth under certain conditions (e.g. in TRAIL-resistant tumors). In contrast to CD95 ligand or TNFα, which cause severe toxic side effects upon systemic administration, TRAIL appears to be a relatively safe and promising candidate for clinical application [27]. However, some concerns about potential toxic side effects on human hepatocytes or brain tissue have been raised, which might be related to the TRAIL preparations used in these studies [32,33].

**Regulation of death receptor signaling in cancer**

**Death receptors and the death-inducing signaling complex**

The idea to specifically target death receptors to trigger apoptosis in tumor cells is attractive for cancer therapy, as death receptors have a direct link to the cell’s death machinery [7]. Also, apoptosis upon death receptor triggering is considered to occur independently of the p53 tumor suppressor gene, which is impaired in the majority of human tumors [34]. However, many tumors remain resistant towards treatment with death-inducing ligands such as TRAIL, despite expression of agonistic TRAIL receptors, which has been related to the dominance of anti-apoptotic signals (e.g. those delivered by nuclear factor-κB, AKT or by IAPs) [7]. Importantly, numerous studies have shown that TRAIL, together with cytotoxic drugs or γ-irradiation strongly synergized to achieve anti-tumor activity in various cancer cell lines and different mouse models of human cancers [7]. The molecular mechanisms that account for this synergistic interaction might include transcriptional upregulation of the agonistic TRAIL receptors TRAIL-R1 and TRAIL-R2. Recent evidence suggest that p53 is crucial for sensitization to TRAIL by chemotherapy through transcriptional upregulation of TRAIL-R2 in some tumors (e.g. mismatch repair-deficient colorectal cancer cells harboring Bax mutations) [35]. Intriguingly, pre-exposure to chemotherapy restored TRAIL sensitivity through a p53-mediated increase of TRAIL-R2 expression even in resistant colorectal carcinoma cells lacking Bax expression, indicating that sequential combination of anticancer agents with TRAIL may overcome some forms of resistance [35]. In addition, the proteasome inhibitor MG132 has recently been reported to upregulate TRAIL-R2 expression and to cooperate with TRAIL to trigger apoptosis even in Bax-deficient colon carcinoma cells [36]. In addition, chemotherapy was found to enhance DISC assembly upon TRAIL receptor triggering in colon carcinoma cells [37]. Moreover, tumor resistance might be caused by loss of death receptor expression function, and by soluble receptors such as soluble CD95 or decoy receptor 3 (DcR3) [27,38,39]. Loss of TRAIL-R1 or -R2 expression might also be caused by epigenetic alterations such as promoter hypermethylation (e.g. in neuroblastoma) [40]. Signaling by death receptors can also be negatively regulated by proteins that associate with their cytoplasmatic domains, such as FLICE-inhibitory protein (FLIP) or phosphoprotein enriched in diabetes/phosphoprotein enriched in astrocytes-15kDa (PED/PEA-15), which are expressed at high levels in many cancers [11,41]. Interestingly, PED/PEA-15 was found to be recruited to the DISC in TRAIL-resistant malignant glioma cells, indicating that it may interfere with caspase-8 activation at the DISC [42]. Importantly, peroxisome proliferator-activated receptor (PPAR)γ ligands or proteasome inhibitors have been reported to selectively reduce FLIP expression, thereby sensitizing tumor cells for TRAIL treatment [43,44]. Furthermore, activation of protein kinase C was found to negatively regulate recruitment of death domain-containing molecules into their respective death receptor-associated signaling complex [45]. Interestingly, a recent study provided evidence that FADD, a cytoplasmic adaptor molecule that bridges the interactions between membrane death receptors and initiator caspases, also resides in the nucleus, where it interacts with methyl-CpG binding domain protein 4, suggesting a novel link between genome surveillance and apoptosis [46].

**Caspases**

Despite the key role of caspases in cell death execution, caspase mutations have only been identified at low frequency in some tumors (e.g. colorectal cancer or head and neck carcinoma) [47,48]. Instead, caspases are frequently inactivated by epigenetic mechanisms [18,49]. Interestingly, co-methylation for caspase-8 and FLIP was observed in neuroblastoma, suggesting that genes are not randomly targeted by methylation in cancer [50]. Importantly, restoration of caspase-8 expression by gene transfer, demethylation treatment or interferon (IFN)-γ sensitized resistant tumor cells for death receptor- or drug-induced apoptosis [18]. IFN-sensitive response elements were subsequently identified in the caspase-8 promoter, showing that IFN-γ could directly activate caspase-8 transcription [51,52].

**Inhibitor of apoptosis proteins**

IAPs such as XIAP, cIAP1, cIAP2, survivin and livin (ML-IAP) are expressed at high levels in many tumors...
and can interfere with death receptor signaling [53,54]. IAPs have been reported to directly inhibit active caspase-3 and -7, and to block caspase-9 activation [53]. Interestingly, recent evidence suggests that direct interaction between survivin and Smac/DIABLO, rather than inhibition of effector caspases, is essential for anti-apoptotic activity of survivin [55]. Also, the antiapoptotic function of survivin has been related to inhibition of apoptotic activity of survivin [55]. Interestingly, recent evidence suggests that direct interaction between survivin and Smac/DIABLO, rather than inhibition of effector caspases, is essential for anti-apoptosis in response to anticancer therapy. These studies should eventually allow identification of novel therapeutic targets, and provide the basis for individual tumor therapy.

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- **of outstanding interest**


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