REVIEW

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Modulation of apoptosis signaling for cancer therapy

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Abstract

Apoptosis, the cell's intrinsic death program, plays a central role in regulating tissue homeostasis. Also, most cytotoxic therapies used for cancer treatment, such as chemotherapy, γ-irradiation, suicide genes, or immunotherapy, predominantly act by triggering apoptosis in target cells. Thus, understanding the molecular events that regulate apoptosis and how tumor cells evade apoptotic deletion have provided a paradigm to link cancer genetics and response to cancer therapy. Therefore, insights into the mechanisms regulating drug-induced apoptosis provide rational targets for novel therapeutic interventions.

Key words: apoptosis, cancer therapy, resistance.

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INTRODUCTION

Apoptosis or programmed cell death is an intrinsic cell death program that is involved in the regulation of various physiological and pathological processes [10]. Apoptosis is critical for tissue homeostasis, which depends on the balance between proliferation and cell death [6]. One of the most important recent advances in cancer research is the recognition that apoptosis plays a major role in both tumor formation and treatment response [4, 11, 13]. Since apoptosis is a gene-directed program, it can be disrupted by genetic mutations [11]. Oncogenic mutations often result in an increased growth rate or a block in apoptosis, leading to tumor initiation, progression, and resistance to current treatment approaches [5]. Defects in apoptosis pathways may create a permissive environment for genetic instability and accumulation of mutations, promote resistance to immune-based deletion, and support survival [4]. Moreover, killing of tumor cells by current anticancer therapies, such as cytotoxic drugs, γ-irradiation, suicide genes or immunotherapy, is mediated by triggering apoptosis in target cells [4]. To this end, elucidation of the core machinery of apoptosis has provided new insight into cancer biology, revealing novel strategies for cancer therapy. The expression profiles of apoptosis-regulating genes in tumors of individual patients, e.g. by microarray or proteome analysis, may serve as predictive markers of drug response.

APOPTOSIS IN CANCER THERAPY

It is now well established that most cytotoxic agents primarily act by triggering apoptosis in cancer cells [4]. This implies that cellular responses occurring after drug-target interaction have a profound impact on drug-induced cytotoxicity. The mechanisms for inducing apoptosis may differ for individual stimuli and have not been exactly defined. However, damage to DNA or to other key signaling molecules appears to be a common initial event [11]. This inciting lesion is then propagated by the cellular stress response and multiple stress-inducible molecules, e.g. JNK, MAPK/ERK, NFκB, or ceramide, may have a profound impact on apoptosis pathways [3]. Since the cytotoxic effects of current therapies are mediated by apoptosis, defects in apoptosis signaling pathways can reduce or abrogate treatment response. Since agents with different primary intracellular targets can trigger apoptosis through similar mechanisms, defects in apoptosis programs may result in multi-drug resistance.

APOPTOSIS SIGNALING PATHWAYS

The majority of apoptosis signaling pathways ultimately result in the activation of caspases, a family of cysteine proteases that act as common death-effector molecules in many forms of cell death [17]. Caspases are

synthesized as inactive zymogens and activated by proteolytic cleavage. The fact that active caspases can activate each other results in amplification of caspase activity through a caspase cascade.

Caspases involved in apoptosis signaling can be categorized into initiator and effector caspases [17]. Initiator caspases transduce various signals into protease activity. Caspase-8 and caspase-10 are directly linked to death-inducing signaling complexes (DISCs) by interacting via their death effector domain with adaptor proteins (FADD) recruited and bound to activated death receptors. Caspase-9 is recruited to the apoptosome via its CARD domain. Effector caspases cleave numerous cytoplasmatic or nuclear substrates which mark many of the morphologic features of apoptotic cell death [10]. For example, polynucleosomal DNA fragmentation is initiated by cleavage of inhibitor of caspase-activated DNase, the inhibitor of the endonuclease caspase-activated DNase that cleaves DNA into the characteristic oligomeric fragments [10]. Likewise, loss of overall cell shape is due to proteolysis of cytosceletal proteins, including fodrin, gelsolin, actin, plectrin, and cytokeratin, while nuclear shrinking and budding occurs after degradation of lamin [10].

The activation of caspases can be initiated at the plasma membrane by death receptor-mediated signaling (receptor pathway) or at the mitochondria (mitochondrial pathway) [10]. Ligation of death receptors of the tumor necrosis factor receptor superfamily, such as CD95 (APO-1/Fas) or TRAIL receptors, leads to receptor aggregation and recruitment of the adaptor molecule Fas-associated death domain (FADD) and caspase-8 to form the DISC [18]. Caspase-8 becomes activated upon recruitment and propagates apoptosis by direct cleavage of downstream effector caspases. The mitochondrial pathway is initiated by the release of apoptogenic factors such as cytochrome c, apoptosis inducing factor (AIF), Smac/Diablo, Omi/HtrA2, endonuclease G, caspase-2 or caspase-9 from the mitochondrial intermembrane space into the cytosol [14]. The release of cytochrome c from mitochondria results in caspase-3 activation through formation of the cytochrome c/Apaf--1/caspase-9-containing apoptosome complex. Smac/ /Diablo and Omi/HtrA2 promote caspase activation by antagonizing the inhibitory effects to inhibitor of apoptosis proteins (IAPs), while AIF and endonuclease G cause large-scale DNA fragmentation and chromatin condensation [14].

Also, there are multiple connections between the receptor and the mitochondrial pathway. Activation of caspase-8 may lead to cleavage of Bid, a BH3 domain--containing protein of the Bcl-2 family, which upon cleavage triggers cytochrome c release from mitochondria, thereby initiating a mitochondrial amplification loop [14]. Moreover, mitochondria-triggered caspase-6 cleavage may feed back to the receptor pathway by cleaving caspase-8 [14].

APOPTOSIS REGULATORS IN CANCER THERAPY

Caspases

Given the important role of caspases as key effector molecules in numerous forms of cell death including drug-induced apoptosis, the ability of chemotherapeutic agents to trigger caspase activation appears to be a crucial determinant of drug response. As a consequence, defects in caspase activation often results in chemoresistance.

First, expression levels of individual caspases may influence their overall activity, since activation of caspases may simply be impaired by deficient expression levels of caspases [16]. Because of the key role of caspases in the execution of cell death, one might expect a high frequency of caspase mutations in tumors. Interestingly, however, screening for mutations in caspases in various human tumors has not revealed a high frequency of genomic aberrations in caspase genes [16]. Instead, caspase expression and function may be impaired by epigenetic changes such as promotor hypermethylation. To this end, caspase-8 expression was reported to be frequently inactivated by hypermethylation of regulatory sequences of the caspase-8 gene in a number of different tumors cells both *in vitro* and also *in vivo* in primary tumor samples [8, 16]. Importantly, restoration of caspase-8 expression by gene transfer or by demethylation also restored sensitivity of resistant tumor cells to apoptosis induction [8]. In addition, enhanced transcription of caspase genes has been reported in response to cytotoxic therapy. Thus, treatment with interferon (IFN)-γ resulted in increased expression of caspase proteins, which was mediated by direct activation of STAT-1, a downstream transcription factor involved in IFN-γ signaling [7].

Inhibitor of apoptosis proteins

The family of endogenous caspase inhibitors called "inhibitor of apoptosis proteins" (IAPs) comprise human analogues such as XIAP, cIAP1, cIAP2, survivin, and livin (ML-IAP). IAPs have been reported to directly inhibit effector caspases such as caspase-3 and caspase-7 [1, 15]. In addition to modulation of apoptosis, IAP members such as survivin have been implicated in the regulation of mitosis [1]. The activity of IAPs are controlled at various levels, e.g. at the transcriptional level by the transcription factor NFκB that can induce expression of cIAP1, cIAP, and XIAP [1, 15]. In addition, IAPs are negatively regulated by caspase-mediated cleavage. Moreover, Smac/Diablo and Omi, proteins that are released from mitochondria into the cytosol upon apoptosis induction, neutralize IAP function through binding to IAPs, thereby displacing them from caspases [14]. Smac agonists sensitized even resistant tumor cells to apoptosis induction and strongly synergized with TRAIL to eradicate established tumors in an orthotopic mouse

model of malignant glioma, indicating that Smac agonists represent novel promising cancer therapeutics [9].

Inhibition of apoptosis by IAPs in response to chemotherapy has been suggested by several experimental studies suggesting that enhanced IAP expression in cancers may contribute to resistance [1, 15].

Bcl-2 proteins

Bcl-2 family proteins play an important role in the regulation of the mitochondrial pathway, which plays an important role in most forms of drug-induced apoptosis [2]. They comprise both anti-apoptotic members, e.g. Bcl-2 and Bcl-X_L, as well as pro-apoptotic molecules, such as Bax, and BH3-domain-only molecules, e.g. Bid [2]. Upon apoptosis induction, proapoptotic Bcl-2 proteins in the cytosol such as Bax translocate to the outer mitochondrial membrane to promote cytochrome c release. This translocation to mitochondria can be triggered by Bcl-2 proteins which have a BH3-domain-only, such as Bid. Bcl-2 or Bcl-X_L block apoptosis by sequestering BH3-domain-only proteins in stable mitochondrial complexes and/or by preventing cytochrome c release from mitochondria [14]. Imbalances in the ratio of anti- and pro-apoptotic Bcl-2 proteins may tip the balance in favor of survival instead of cell death [2]. Altered expression of Bcl-2 family proteins have been reported in a variety of human cancers and has been linked to treatment response [2].

CASPASE-INDEPENDENT AND NONAPOPTOTIC MODES OF CELL DEATH

Although numerous studies indicate an essential role of caspase-dependent apoptosis in mediating drug response, this concept has also been challenged. So far, a consistent link between cells' ability to undergo apoptosis and drug response could not be observed. Thus, nonapoptotic modes of cell death, e.g. necrosis or some forms of cell death that cannot be easily classified at present, may also mediate response to cytotoxic therapy [12]. However, the relative contribution of these different modes of cell death for treatment response *in vitro* and *in vivo* has not yet been exactly defined.

CONCLUSION

A vast variety of reports over the last years have provided convincing evidence that cell death by apoptosis plays a crucial role in the surveillance of tumor formation and in cytotoxic therapies which primarily act by inducing apoptosis in tumor cells. However, many questions have not yet been answered and are subject to ongoing research. For example, most of the apoptosis signaling molecules have not yet been assessed in clinical samples. Future studies on the role of apoptosis-regulating genes in individual tumors both *in vitro* and *in vivo* in tumor cells of patients under chemotherapy, e.g. by DNA microarrays or proteomic studies, may provide the basis for "tailored" tumor therapy and may identify new targets for therapeutic interventions.

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REFERENCES

1. Altieri D. C. (2003): Validating survivin as a cancer therapeutic target. Nat. Rev. Cancer, **3**, 46–54.
2. Cory S. and Adams J. M. (2002): The Bcl2 family: regulators of the cellular life-or-death switch. Nat. Rev. Cancer, **2**, 647–656.
3. Davis R. J. (2000): Signal transduction by the JNK group of MAP kinases. Cell, **103**, 239–252.
4. Debatin K. M. and Krammer P. H. (2004): Death receptors in chemotherapy and cancer. Oncogene, **23**, 2950–2966.
5. El-Deiry W. S. (2001): Insights into cancer therapeutic design based on p53 and TRAIL receptor signaling. Cell Death Differ., **8**, 1066–1075.
6. Evan G. I. and Vousden K. H. (2001): Proliferation, cell cycle and apoptosis in cancer. Nature, **411**, 342–348.
7. Fulda S. and Debatin K. M. (2002): IFNgamma sensitizes for apoptosis by upregulating caspase-8 expression through the Stat1 pathway. Oncogene, **21**, 2295–2308.
8. Fulda S., Kufer M. U., Meyer E., van Valen F., Dockhorn-Dworniczak B. and Debatin K. M. (2001): Sensitization for death receptor- or drug-induced apoptosis by re-expression of caspase-8 through demethylation or gene transfer. Oncogene, **20**, 5865–5877.
9. Fulda S., Wick W., Weller M. and Debatin K. M. (2002): Smac agonists sensitize for Apo2L/TRAIL- or anticancer drug-induced apoptosis and induce regression of malignant glioma *in vivo*. Nat. Med., **8**, 808–815.
10. Hengartner M. O. (2000): The biochemistry of apoptosis. Nature, **407**, 770–776.
11. Johnstone R. W., Ruefli A. A. and Lowe S. W. (2002): Apoptosis: a link between cancer genetics and chemotherapy. Cell, **108**, 153–164.
12. Leist M. and Jaattela M. (2001): Four deaths and a funeral: from caspases to alternative mechanisms. Nat. Rev. Mol. Cell Biol., **2**, 589–598.
13. Lowe S. W. and Lin A. W. (2000): Apoptosis in cancer. Carcinogenesis, **21**, 485–495.
14. Saelens X., Festjens N., Vande Walle L., van Gurp M., van Loo G. and Vandenabeele P. (2004): Toxic proteins released from mitochondria in cell death. Oncogene, **23**, 2861–2874.
15. Salvesen G. S. and Duckett C. S. (2002): IAP proteins: blocking the road to death's door. Nat. Rev. Mol. Cell Biol., **3**, 401–410.
16. Teitz T., Wei T., Valentine M. B., Vanin E. F., Grenet J., Valentine V. A., Behm F. G., Look A. T., Lahti J. M. and Kidd V. J. (2000): Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. Nat. Med., **6**, 529–535.
17. Thornberry N. A. and Lazebnik Y. (1998): Caspases: enemies within. Science, **281**, 1312–1316.
18. Walczak H. and Krammer P. H. (2000): The CD95 (APO-1/Fas) and the TRAIL (APO-2L) apoptosis systems. Exp. Cell Res., **256**, 58–66.