



# Advances in the Application of Apoptotic Proteins and Alternative Splicing in Tumor Therapy: A Narrative Review

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## Abstract

An apoptosis-resistant state determined by apoptotic protein expression is commonly seen in the initiation, progression, and treatment failure stages of human cancer, and anti-tumor drugs targeting apoptotic proteins have been increasingly developed over the past three decades. However, the frequently alternative splicing of apoptotic proteins diminished the ability of targeting drugs to bind to apoptotic proteins and, consequently, limit the drug efficacy. Currently, accumulating evidence has demonstrated that many alternative splicing events have been associated to apoptosis resistance in different cancers. Therefore, the intervention targeting alternative splicing for regulating tumor cell apoptosis is expected to become a new strategy and new direction of antitumor therapy. Here, we present well established alternative splicing events that occur in different apoptosis-related genes and their modification by several approaches with cancer therapeutic purposes.

**Keywords:** Apoptosis; Alternative splicing; Cancer; Therapeutics

## Introduction

Apoptosis is a biological phenomenon of normal human development, plays an important role in removing dangerous cells from the body and is one of the natural ways in which living organisms avoid the occurrence and progression of tumors (1). The apoptosis process has obvious dysfunction during the occurrence and development of various tumors, and tumor cells evolve a series of biological functions to limit or escape from apoptosis (2). For more than 30 years, clinical oncology

has focused on antitumor therapies that promote apoptosis to eliminate cancer cells and have obtained certain breakthroughs. However, due to the limited bioavailability, stability, tumor penetration, nonmalignant tissue toxicity, drug–drug interactions, and off-target effects of proapoptotic drugs, the efficacy of antitumor drugs based on promoting tumor cell apoptosis is still not ideal in clinical application (3, 4).



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Alternative splicing of apoptotic proteins is an important regulatory event involved in apoptosis. Pre-messenger RNAs (mRNAs) of apoptotic factors can be processed into transcripts with different structures through alternative splicing to encode different apoptotic protein isoforms that exert different apoptotic functions. In recent years, studies exploring tumor therapies that disrupt the alternative splicing of apoptotic factors have also made some progress. This study summarizes the advances in apoptotic proteins and their alternative splicing in tumor therapy.

#### *Apoptotic pathways and apoptosis-related factors*

##### *Death receptor pathways and related proteins in apoptosis*

Death receptors are a group of cell-surface markers in the TNFR superfamily can expose their death domains (DDs) by binding to their corresponding ligands and undergoing oligomerization and conformational changes. DDs can recruit adaptor proteins to further activate downstream caspase signaling cascades and induce apoptosis (5). Death receptors mainly include the following factors: Fas, TNF- $\alpha$ , TNF-related apoptosis-inducing ligand (TRAIL), death receptor 3 (DR3), death receptor 4 (DR4), and death receptor 5 (DR5). Fas first binds with its ligand FasL to undergo the trimerization, and then Fas is activated. Activated Fas exposes its DD, and DD recruits adaptor proteins and induces a conformational change in Fas-associated with death domain protein (FADD) to activate FADD. Activated FADD activates caspase-8 through death effector domain (6).

##### *Mitochondrial pathway and related proteins in apoptosis*

The mechanism of the mitochondrial pathway of apoptosis mainly involves mitochondria undergoing mitochondrial outer membrane permeabilization (MOMP) to cause release of cytochrome  $c$  to initiate the apoptosis program when cells are stimulated by oncogene activation, DNA damage, hypoxia, or loss of growth factors. MOMP and

cytochrome  $c$  release are the critical steps in the intracellular apoptotic pathways, whereas Bcl-2 protein family members are the major regulatory factors of these steps (7). The apoptosis-related proteins in the Bcl-2 family are divided into antiapoptotic proteins and proapoptotic proteins. The antiapoptotic proteins mainly include Bcl-2, Bcl-w, Mcl-1, Bcl-xL, A1, Boo, and Ced-9, whereas the proapoptotic proteins mainly include Bok, Bcl-xS, Bax, Bak, Bid, Bad, and Egl-1 (8).

#### **Apoptotic factors and tumor therapy**

##### *Tumor therapies targeting death receptor pathways (Fig.1)*

As mentioned above, TNF-superfamily death receptors are the core of exogenous apoptotic pathways in cells and are critical proapoptotic factors. Therefore, they are potential drug targets to promote the apoptosis of tumor cells to achieve antitumor effects. Among TNF-superfamily death receptors, the TRAIL receptor is currently considered the most promising drug target for promotion of tumor cell apoptosis (9). TRAIL is a transmembrane trimeric glycoprotein that can bind to death receptors DR4 and DR5 to induce the trimer formation in the intracellular DDs of these receptors to further recruit FADD and activate downstream caspase-8, -3, and -7, thereby inducing tumor cell apoptosis (10). In addition, activated caspase-8 can activate the endogenous mitochondrial apoptotic pathway by cleaving the Bcl-2 family member Bid to amplify further the apoptotic signal. Based on these mechanisms, proapoptotic agonists that can bind to TRAIL, DR4, and DR5 have been developed in recent years, and they can directly activate exogenous apoptotic pathways to induce tumor cell apoptosis and achieve antitumor effects (10, 11). Over the past decade, studies have focused on the development of TRAIL agonists. Around the year of 2000, researchers studied recombinant human TRAIL-activating monoclonal antibodies as a single drug or combined with chemotherapy drugs or rituximab to treat patients with solid tumors or hematologic malignancies (12).

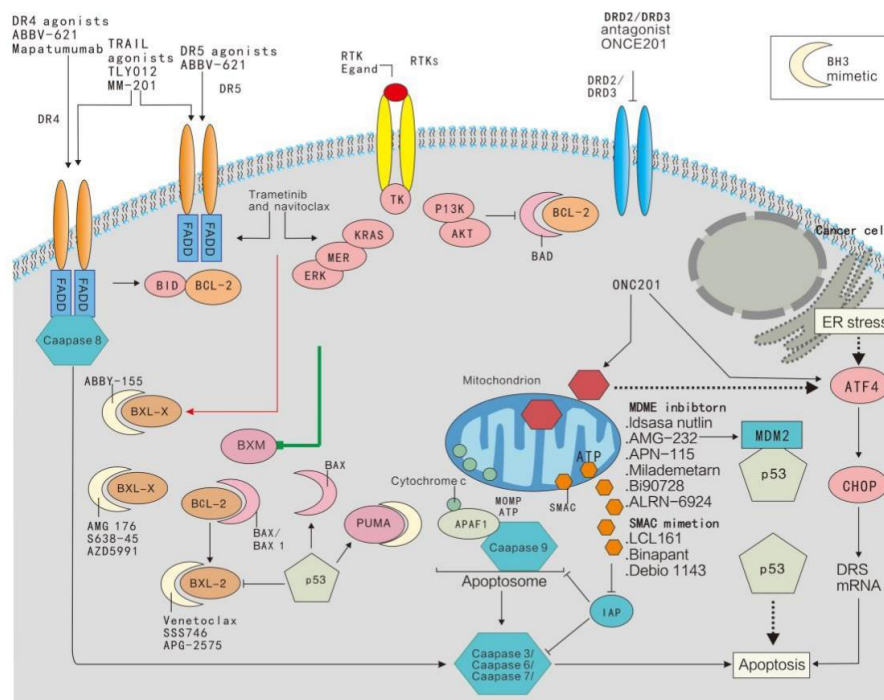


Fig. 1: Therapeutic approaches targeting apoptosis pathways in cancer cells (3)

However, due to their short half-lives, limited ability to induce receptor aggregation, and lack of sensitive markers for death receptor binding, the expected tumor therapy effect has not been observed in clinical trials (13). Furthermore, due to genetic variations in the expression levels of DR4 and DR5, the posttranslational protein modification of death receptors, and the reduction in cell-surface receptor expression and/or density, the application of TRAIL-activating monoclonal antibodies in clinical practice is limited (12-14). ONC201, a small molecule containing a three-ring pyridone structure, can bind to dopamine receptors DRD2 and DRD4 and mitochondrial proteases to induce DR5-dependent apoptosis (15, 16). Further clinical trials have confirmed that after the treatment with TRAIL-activating monoclonal antibody in preclinical models of endometrial cancer and breast cancer, the ONC201 antitumor activity is significantly enhanced, which may result from the initiation of TRAIL-induced apoptosis of tumor cells medi-

ated by ONC201 (17, 18). MM-201 is a fusion protein combining IgG1 and a single chain TRAIL, and preclinical experimental results show that it has excellent antitumor activities. TRAIL polyethylene glycol formula with extended half-life and stability has been developed for treating liver fibrosis and may be tested in cancer patients (19). In short, a number of research data have reopened the prospects of tumor therapies based on TRAIL agonist to promote tumor cell apoptosis (20). Further clinical trial verification and evaluation are still needed for full clinical application. DR4 and DR5 monoclonal antibodies (such as mapatumumab, lexatumumab, conatumumab, tigatuzumab, and drozitumab) have a strong activity of recruiting and activating receptors because of their long half-lives (21-24). Furthermore, DR4 and DR5 agonists exhibit high specificity and selectivity to cancer cells and do not damage nonmalignant tissues, so they were initially considered promising alternatives to soluble receptor agonists. However, DR4 agonists or

DR5 agonists alone still show limited clinical efficacy when targeting different tumors and in different individuals. Mapatumumab is a DR4-activating antibody that has shown excellent tolerance in clinical trials, but it has exhibited limited clinical efficacy in the chemotherapy of patients with non-small-cell lung cancer, colorectal cancer, and other solid tumors (25, 26). The DR5-activating antibody lexatumumab has been used for late-stage solid tumor patients as a single drug or combined with chemotherapy drugs, and long-lasting and stable treatment effects have been found in childhood osteosarcoma and adult sarcoma patients (27, 28). Other DR5 agonists, including conatumumab, tigatuzumab, LBY135, and drozitumab, have exhibited certain clinical responsiveness when combined with 5-fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6). Since this group showed no significance difference compared to the tumor treatment group, researchers have put forward the argument that DR5 agonists only have a placebo effect (29).

#### *Tumor therapies targeting mitochondrial pathway members*

The Bcl-2 protein family is the core protein group regulating the intracellular mitochondrial apoptotic pathway to initiate the apoptosis program. Therefore, targeting proteins of this family to control tumor growth by promoting apoptosis has become a hotspot in tumor research. This group of regulatory drugs mainly includes drugs that inhibit antiapoptotic Bcl-2 family members and drugs that enhance the activity of proapoptotic Bcl-2 family members. Recent studies on inhibitors targeting antiapoptotic members in the Bcl-2 protein family have obtained breakthrough progress as detailed below:

**BH3 mimetics.** BH3 mimetics refer to the tumor therapy strategy of synthesizing small-molecule BH3 mimetics to bind to antiapoptotic proteins in the Bcl-2 family to induce apoptosis. For example, the ABT-737 small molecule can selectively bind to the hydrophobic domain of Bcl-2, Bcl-xL, or Bcl-W to promote the interaction between the hydrophobic pocket region and proapoptotic

BH3-containing proteins to exert proapoptotic effects. ABT-737 used as a single drug or combined with radiotherapy and paclitaxel in lymphoma and small-cell lung cancer mouse xenograft models shows significant tumor cell growth inhibition effects (30, 31)). Second-generation drugs, such as navitoclax, show an antitumor response in 34.6% of patients with refractory chronic lymphocytic leukemia (CLL), and the overall response rate reaches 70% when combined with rituximab. However, the antitumor response of navitoclax used as a single drug or in combination is still not clear in patients with late-stage solid tumors (32). The Bcl-2 selective inhibitor venetoclax can significantly inhibit the growth of CLL and non-Hodgkin lymphoma cells in mouse xenograft models. Based on the above research evidence, venetoclax was approved as the first-line treatment drug for CLL patients in May 2019. However, the antitumor effect of venetoclax in solid tumors is still under exploration (33, 34). Selective Bcl-xL inhibitors have currently been developed and are mainly used in solid tumor therapy. For example, ABBV-155 is one antitumor drug that binds specifically to Bcl-xL. A phase I trial is underway on the safety and pre-activity of patients with refractory solid tumors. The tumor vaccine Bcl-xL<sub>42</sub>-CAF09b, targeting Bcl-xL, is also in a phase I trial for prostate cancer patients (3).

**BAX agonists.** BAX is an important proapoptotic protein in the Bcl-2 family. Small molecules designed and synthesized using the BAX Ser184 regulatory site as the target can induce apoptosis through localization and insertion into the mitochondrial membrane. They have had a positive antitumor effect in a lung cancer mouse model. The BAX agonists SMBA1 and SMBA3 can specifically bind to BAX to inhibit phosphorylation of its Ser184 residue and promote its oligomerization, leading to cytochrome *c* release and apoptosis (35, 36). Furthermore, SMBA1 can be used in combination with the compounds CYD-4-61 and GL-0383, and their activity of inducing tumor cell apoptosis and inhibiting tumor cell proliferation has been observed in lung cancer mouse xenograft models and breast cancer cell lines (37).

Other BAX agonists, such as bam7 and BTSA1, exhibit excellent antitumor activities in glioblastoma and acute myeloid leukemia (AML) cell lines (38, 39).

**BIM agonists.** BIM is a core proapoptotic protein for BAX activation and is a bridge between the cellular endogenous mitochondrial apoptotic pathway and other signaling pathway kinases (such as ERK1/2, Akt1, c-Jun N-terminal kinase, and JUN). ERK1/2 and MAPK1 can promote BIM phosphorylation to cause its proteasome degradation, further inhibiting the biological function of BIM on BAX activation and increase the cell survival capacity (40). This tumor cell survival mechanism has been confirmed in studies on various tumors, including NSCLC and chronic myeloid leukemia. Preclinical studies indicate that BIM without the BH3 domain could make tumors resistant to epidermal growth factor receptor EGFR tyrosine kinase inhibitors (TKIs) (41). NSCLC patients with this germline polymorphism have a poor clinical prognosis when treated with gefitinib (EGFR TKI) or crizotinib (ALK or ROS1 that targeted TKI to alter NSCLC) (42, 43). In addition, cytotoxic drug treatment upregulates BIM expression and promotes chemotherapy-mediated tumor cell apoptosis. The SYK/JAK inhibitor cerdulatinib also upregulates BIM expression and exhibits synergistic effects with venetoclax (44). The above results have inspired research on promoting tumor cell apoptosis targeting BIM.

**IAP inhibitors.** Inhibitor of apoptosis proteins (IAPs) are often overexpressed in various malignant tumors and are closely associated with poor tumor prognosis. Among the eight human IAP proteins, XIAP, IAP1, IAP2, and baculovirus IAP repeat-containing protein 7 (usually referred as ML-IAP) have obvious antiapoptotic activity. Many recent studies have confirmed that IAP protein inhibitors are potential antitumor drugs to induce tumor cell apoptosis. Several IAP antagonists, including small molecules and oligonucleotides, have been used in clinical trials. However, they have not been approved by the FDA (45, 46). Smac and HTRA2 can be released from mitochondria and selectively bind to XIAP to

antagonize the inhibitory effect of XIAP on caspase-3, -7, and -9 and thereby block the inhibition of apoptosis by XIAP (47). The IAP inhibitor CUDC-427 exhibited an excellent antitumor effect in a phase I trial, but it provoked strong adverse reactions, such as fatigue, nausea, vomiting, and rash (48). The IAP inhibitor LCL161 promotes inflammatory responses by upregulating interferon signals to have antitumor activity in patients with refractory multiple myeloma (49). Novel targeted drugs targeting apoptotic pathways are continuously developing, and some drugs have great potential because of their high levels of tumor selectivity. However, the development of antitumor treatments targeting apoptotic pathways is still in the stage of exploration and evaluation, and antitumor drugs targeting apoptotic pathways have limited effects in certain tumors, especially solid tumors. Antitumor drugs targeting one apoptotic pathway or combination regimes still cannot avoid adverse reactions. The high heterogeneity of tumors and the high-frequency alternative splicing during apoptotic protein expression reduce the ability of targeted drugs to bind to apoptotic proteins and limit the antitumor effects of apoptotic protein inhibitors. In the future, the development of drugs targeting apoptotic pathways should actively seek to overcome the limitations on the application of targeted drugs, such as tumor heterogeneity and the high frequency of alternative splicing of apoptotic factors (3, 50).

#### *Tumor therapies targeting the alternative splicing of apoptotic factors*

As mentioned above, abnormal alternative splicing of cancer-related factors is involved in the occurrence and progression of tumors, including tumor invasion, metastasis, apoptosis and proliferation. The regulation and disruption of alternative splicing may become a new research hotspot in cancer therapy.

#### *Disruption of alternative splicing by targeting splicing regulatory proteins*

Overexpression and functional changes of various alternative splicing regulators (SRs) are criti-

cal factors inducing abnormal alternative splicing of tumor-related factors to promote further tumor occurrence and development. SRs have become novel targets of cancer therapy. Regulation of protein phosphorylation has become a potential method for regulating splicing by changing the functions of SRs (51). For example, amiloride can inhibit SR protein functions to change the splicing patterns of oncogenes, such as apoptotic peptidase activating factor (APAF1), CT10 regulator of kinase (CRK), Bcl-X, homeodomain interacting protein kinase 3 (HIPK3), and RON/MISTR1 (52). Furthermore, metabolites and their derivatives of some actinomycetes and filamentous fungi can be used as splicing inhibitors to influence tumor cell proliferation, and clinical studies show that they have significantly lower drug toxicity than other chemotherapy drugs. The splicing inhibitor splicestatin A (SSA) is a derivative of the natural product FR901464 of *Pseudomonas*. In breast cancer cell lines, SSA can bind to U2 snRNP to inhibit its biological functions as a splicing protein, and SSA interacts with the splicing factor SF3B subunit SAP145 to arrest breast cancer cells at G1 and G2/M phases (53). Pladienolide B is the derivative of the natural product Mer11107 of *Streptomyces*, and can interact with SF3B to modify U2 snRNP and arrest cervical cancer cells at G1 and G2/M (54). Application of the above molecules targeting SRs in tumor therapy still requires further preclinical studies and clinical validation.

#### *Intervention into alternative splicing by using oligonucleotides*

Because abnormal alternative splicing is an important specific pathological event during tumor occurrence and development, scientists have explored oligonucleotide-based antitumor treatments targeting alternative splicing events. Oligonucleotides can regulate alternative splicing to repair defectively expressed mRNAs or regulate tumor-associated alternative splicing to restore the production of some proteins or even promote the production of new functional proteins (55, 56). For example, some exons are easily spliced to cause abnormal splicing during tran-

scription because they contain exonic splicing silencer (ESS) sequences or are adjacent to intronic splicing silencer (ISS) sequences. Therefore, splicing inhibitors can be suppressed using anti-sense oligonucleotides to prevent loss of exon sequences from the pre-mRNA (57). In addition, application of antisense oligonucleotides to bind to exon2 of the Bcl-x pre-mRNA could inhibit Bcl-xL expression and induce the expression of the proapoptotic protein Bcl-xS to achieve the effect of inducing tumor cell apoptosis and inhibiting tumors (58). The antisense oligonucleotide mipomersen can promote murine double minute (MDM) exon 6 skipping to reduce MDM4 expression and can further inhibit the cell cycle, promote apoptosis, and inhibit melanoma cell growth through p53-dependent signaling pathways (59). Antitumor therapy using oligonucleotide-based regulation of alternative splicing seems to be promising, but despite decades of effort, no oligonucleotide antitumor therapy has been approved by the FDA.

Alternative splicing perturbation is an important biomarker of occurrence and progression of tumors, and intervening in alternative splicing is promising as a type of anticancer therapy. However, tumor therapy targeting alternative splicing is limited by high heterogeneity of tumors and the quantitative trait locus of different ethnic groups. Therefore, continuous research and exploration are required to transition from theoretical mechanism research to clinical application.

#### **Conclusion**

Targeted tumor cell apoptosis is an effective antitumor strategy. However, due to tumor heterogeneity, adverse reactions, and a high frequency of alternative splicing of apoptotic factors, tumor-targeting drugs have limited effects on apoptotic pathways in various tumor types, especially in solid tumors. Therefore, using alternative splicing as the intervention target for regulating tumor cell apoptosis is expected to become a new strategy and new direction of antitumor therapy. Tumor therapy disrupting or directing alternative

splicing to induce tumor cell apoptosis is promising, but more in-depth research and exploration are needed.

## Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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