Iran J Public Health, Vol. 52, No.7, Jul 2023, pp.1311-1319

Review Article

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

Jin He [1#], Weitao Qiu [2#], Yonghong Li [1], Chaojun Wei [1], Zhongtian Bai [3], Jing Jia [1], *Hui Cai [1,4]

1. *NHC Key Laboratory of Diagnosis and Therapy of Gastrointestinal Tumor, Gansu Provincial Hospital, Lanzhou 730000, China*
2. *Gansu Provincial Maternity and Child-Care Hospital, Lanzhou 730050, China*
3. *The Second Department of General Surgery, Lanzhou University First Hospital, Lanzhou 730000, China*
4. *Key Laboratory of Molecular Diagnostics and Precision Medicine for Surgical Oncology in Gansu Province, Gansu Provincial Hospital, Lanzhou 730000, China*

***Corresponding Author:** Email: caialonteam@163.com

(Received 15 Jan 2023; accepted 18 Apr 2023)
#These authors are equally contributed as first authors

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).

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-α, 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 programwhen 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 ofthese 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 proapoptoticproteins 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 glycoproteinthatcan bind to death receptors DR4 and DR5 to induce the trimer formation in the intracellular DDs of these receptors tofurther 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).

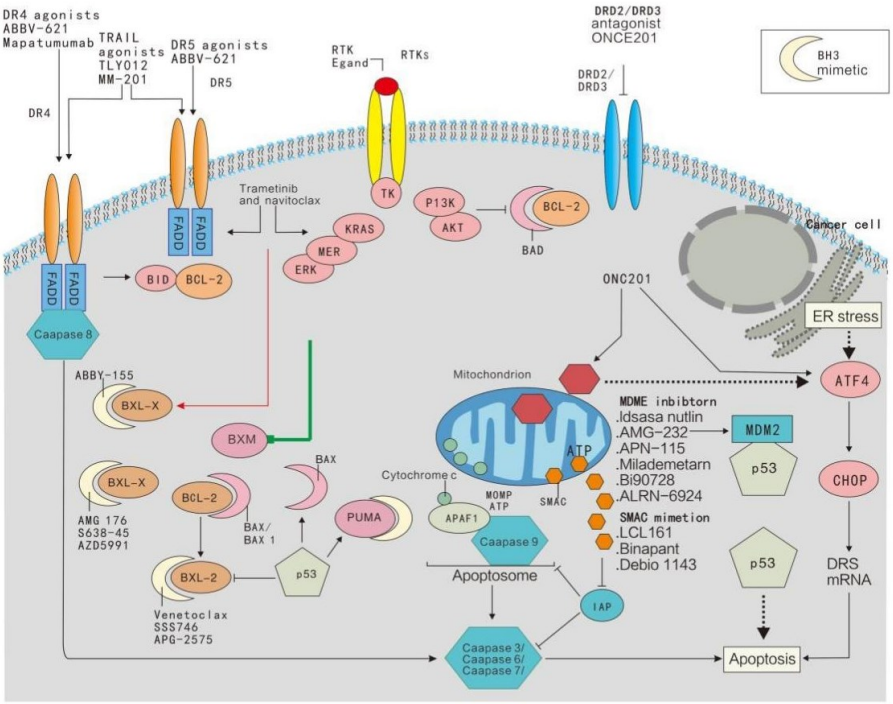

Fig. 1: Therapeutic approaches targeting apoptosis pathways in cancer cells (3)

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 incell-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 mediated 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-fluouracil, 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 mimeticsrefer to the tumor therapy strategy of synthesizing small-molecule BH3 mimeticsto bind to antiapoptotic proteins in the Bcl-2 family toinduce apoptosis. For example, the ABT-737 small molecule can selectively bind to the hydrophobic domain of Bcl-2, Bcl-xL, orBcl-W to promote the interaction between the hydrophobic pocket region and proapoptotic BH-3-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,showan antitumor response in 34.6% of patients with refractory chronic lymphocytic leukemia (CLL), andthe 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 cellsin mouse xenograft models. Based on the above research evidence, venetoclax was approved as the first-line treatment drug for CLL patents 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_42-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 itsSer184 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 itsproteasome 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 apoptosisproteins (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 multiplemyeloma (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 bychanging 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 lowerdrug 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 transcription 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 thepre-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.

Acknowledgements

We thank the National Natural Science Foundation (grant no. 82160534, 82060666), Gansu Provincial Natural Science Foundation of China (grant no. 21JR7RA630,20JR10RA401), the Fundamental Research Funds for the Central Universities (lzujbky-2021-ey06), The 2021 Central-Guided Local Science and Technology Development Fund (grant no.ZYYDDFFZZJ-1) for their financial support. Jin HE and Weitao QIU contributed equally to this work and share first authorship.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. von Schwarzenberg K, Vollmar A (2013). Targeting apoptosis pathways by natural compounds in cancer: marine compounds as lead structures and chemical tools for cancer therapy. *Cancer Letters,* 332:295-303.
2. Ichim G, Tait S (2016). A fate worse than death: apoptosis as an oncogenic process. *Nat Rev Cancer,* 16(8):539-48.
3. Carneiro B, El-Deiry W (2020). Targeting apoptosis in cancer therapy. *Nat Rev Clin Oncol,* 17(7):395-417.
4. Pentimalli F, Grelli S, Di Daniele N, Melino G, Amelio I (2019). Cell death pathologies: targeting death pathways and the immune system for cancer therapy. *Genes Immun,* 20:539-554.
5. MacFarlane M (2003). TRAIL-induced signalling and apoptosis. *Toxicol Lett,* 139(2-3):89-97.
6. Pećina-Slaus N (2009). [Genetic and molecular insights into apoptosis]. *Acta Med Croatica* ,2:13-9.
7. Kalkavan H, Green D (2018). MOMP, cell suicide as a BCL-2 family business. *Cell Death Differ,* 25(1):46-55.
8. Singh R, Letai A, Sarosiek K (2019). Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. *Nature Reviews Molecular Cell Biology,* 20:175-193.
9. Yuan X, Gajan A, Chu Q, Xiong H, Wu K, Wu G (2018). Developing TRAIL/TRAIL death receptor-based cancer therapies. *Cancer Metastasis Rev,* 37(4):733-748.
10. Emery J, McDonnell P, Burke M, et al (1998). Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. *J Biol Chem,* 273(23):14363-7.
11. Falschlehner C, Ganten T, Koschny R, Schaefer U, Walczak H (2009). TRAIL and other TRAIL receptor agonists as novel cancer therapeutics. *Adv Exp Med Biol,* 647:195-206.
12. Herbst R, Kurzrock R, Hong D, et al (2010). A first-in-human study of conatumumab in adult patients with advanced solid tumors. *Clin Cancer Res* ,16(23):5883-91.
13. Amm H, Oliver P, Lee C, Li Y, Buchsbaum D (2011). Combined modality therapy with TRAIL or agonistic death receptor antibodies. *Cancer Biol Ther,* 11(5):431-49.
14. Quintavalle C, Condorelli G (2012). Dulanermin in cancer therapy: still much to do. *Transl Lung Cancer Res,* 1(2):158-9.
15. Ralff M, Kline C, Küçükkase O, et al (2017). ONC201 Demonstrates Antitumor Effects in Both Triple-Negative and Non-Triple-Negative Breast Cancers through TRAIL-Dependent and TRAIL-Independent Mechanisms. *Mol Cancer Ther,* 16(7):1290-1298.
16. Wang J, Wang H, Wang L, et al (2016). Silencing the epigenetic silencer KDM4A for TRAIL and DR5 simultaneous induction and antitumor therapy. *Cell Death Differ,* 23(11):1886-1896.
17. Wagner J, Kline C, Zhou L, et al (2018). Dose intensification of TRAIL-inducing ONC201 inhibits metastasis and promotes intratumoral

NK cell recruitment. *J Clin Invest,* 128(6):2325-2338.
18. Stein M, Malhotra J, Tarapore R, et al (2019). Safety and enhanced immunostimulatory activity of the DRD2 antagonist ONC201 in advanced solid tumor patients with weekly oral administration. *J Immunother Cancer,* 7(1):136.
19. Park J, Oh Y, Park Y, et al (2019). Targeting of dermal myofibroblasts through death receptor 5 arrests fibrosis in mouse models of scleroderma. *Nat Commun,* 10(1):1128.
20. Holland P (2014). Death receptor agonist therapies for cancer, which is the right TRAIL? *Cytokine Growth Factor Rev,* 25(2):185-93.
21. Zhang S, Zheng C, Zhu W, et al (2019). A novel anti-DR5 antibody-drug conjugate possesses a high-potential therapeutic efficacy for leukemia and solid tumors. *Theranostics,* 9(18):5412-5423.
22. Dubuisson A, Favreau C, Fourmaux E, et al (2019). Generation and characterization of novel anti-DR4 and anti-DR5 antibodies developed by genetic immunization. *Cell Death Dis,* 10(2):101.
23. Brünker P, Wartha K, Friess T,et al (2016). RG7386, a Novel Tetravalent FAP-DR5 Antibody, Effectively Triggers FAP-Dependent, Avidity-Driven DR5 Hyperclustering and Tumor Cell Apoptosis. *Mol Cancer Ther,* 15(5):946-57.
24. Milutinovic S, Kashyap A, Yanagi T, et al (2016). Dual Agonist Surrobody Simultaneously Activates Death Receptors DR4 and DR5 to Induce Cancer Cell Death. *Mol Cancer Ther,* 15(1):114-24.
25. von Pawel J, Harvey J, Spigel D,et al (2014). Phase II trial of mapatumumab, a fully human agonist monoclonal antibody to tumor necrosis factor-related apoptosis-inducing ligand receptor 1 (TRAIL-R1), in combination with paclitaxel and carboplatin in patients with advanced non-small-cell lung cancer. *Clin Lung Cancer,* 15(3):188-196.e2.
26. Hotte S, Hirte H, Chen E, et al (2008). A phase 1 study of mapatumumab (fully human monoclonal antibody to TRAIL-R1) in patients with advanced solid malignancies. *Clin Cancer Res ,* 14(11):3450-5.
27. Plummer R, Attard G, Pacey S, et al (2007). Phase 1 and pharmacokinetic study of lexatumumab in patients with advanced cancers. *Clin Cancer Res,* 13(20):6187-94.
28. Smith M, Jin F, Joshi I (2007). Bortezomib sensitizes non-Hodgkin's lymphoma cells to apoptosis induced by antibodies to tumor necrosis factor related apoptosis-inducing ligand (TRAIL) receptors TRAIL-R1 and TRAIL-R2. *Clin Cancer Res ,*13(18 Pt 2):5528s-5534s.
29. Paz-Ares L, Bálint B, de Boer R, et al (2013). A randomized phase 2 study of paclitaxel and carboplatin with or without conatumumab for first-line treatment of advanced non-small-cell lung cancer. *J Thorac Oncol,* 8(3):329-37.
30. Oltersdorf T, Elmore S, Shoemaker A,et al (2005). An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature,* 435(7042):677-81.
31. Del Gaizo Moore V, Brown J, Certo M, Love T, Novina C, Letai A (2007). Chronic lymphocytic leukemia requires BCL2 to sequester prodeath BIM, explaining sensitivity to BCL2 antagonist ABT-737. *J Clin Invest,* 117(1):112-21.
32. Roberts A, Seymour J, Brown J, et al (2012). Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. *Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology,* 30:488-96.
33. Fischer K, Al-Sawaf O, Bahlo J, et al (2019). Venetoclax and Obinutuzumab in Patients with CLL and Coexisting Conditions. *N Engl J Med,* 380(23):2225-2236.
34. Seymour J, Kipps T, Eichhorst B, et al (2018). Venetoclax-Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. *N Engl J Med,* 378(12):1107-1120.
35. Xin M, Li R, Xie M, et al (2014). Small-molecule Bax agonists for cancer therapy. *Nat Commun,* 5:4935.
36. Li R, Ding C, Zhang J, et al (2017). Modulation of Bax and mTOR for Cancer Therapeutics. *Cancer Res,* 77(11):3001-3012.
37. Gavathiotis E, Reyna D, Bellairs J, Leshchiner E, Walensky L (2012). Direct and selective small-molecule activation of proapoptotic BAX. *Nat Chem Biol,* 8(7):639-45.
38. Reyna D, Garner T, Lopez A, et al (2017). Direct Activation of BAX by BTSA1 Overcomes Apoptosis Resistance in Acute Myeloid Leukemia. *Cancer cell,* 32(4):490-505.e10.

39. Garner T, Amgalan D, Reyna D, Li S, Kitsis R, Gavathiotis E (2019). Small-molecule allosteric inhibitors of BAX. *Nat Chem Biol,* 15(4):322-330.
40. Weston C, Balmanno K, Chalmers C, et al (2003). Activation of ERK1/2 by deltaRaf-1:ER* represses Bim expression independently of the JNK or PI3K pathways. *Oncogene,* 22(9):1281-93.
41. Xia J, Bai H, Yan B, Li R, Shao M, Xiong L, Han B (2017). Mimicking the BIM BH3 domain overcomes resistance to EGFR tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer. *Oncotarget,* 8(65):108522-108533.
42. Costa D, Halmos B, Kumar A, et al (2007). BIM mediates EGFR tyrosine kinase inhibitor-induced apoptosis in lung cancers with oncogenic EGFR mutations. *PLoS Med,* 4(10):1669-79; discussion 1680.
43. Ng K, Hillmer A, Chuah C, et al (2012). A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. *Nat Med,* 18(4):521-8.
44. Prukova D, Andera L, Nahacka Z,et al (2019). In VivoCotargeting of BCL2 with Venetoclax and MCL1 with S63845 Is Synthetically Lethal in Relapsed Mantle Cell Lymphoma. *Clin Cancer Res ,* 25(14):4455-4465.
45. Fulda S, Vucic D (2012). Targeting IAP proteins for therapeutic intervention in cancer. *Nat Rev Drug Discov,* 11(2):109-24.
46. Gyrd-Hansen M, Meier P (2010). IAPs: from caspase inhibitors to modulators of NF-kappaB, inflammation and cancer. *Nat Rev Cancer,* 10(8):561-74.
47. Vucic D, Franklin M, Wallweber H, et al (2005). Engineering ML-IAP to produce an extraordinarily potent caspase 9 inhibitor: implications for Smac-dependent anti-apoptotic activity of ML-IAP. *Biochem J,* 385(Pt 1):11-20.
48. Tolcher A, Bendell J, Papadopoulos K, et al (2016). A Phase I Dose-Escalation Study Evaluating the Safety Tolerability and Pharmacokinetics of CUDC-427, a Potent, Oral, Monovalent IAP Antagonist, in Patients with Refractory Solid Tumors. *Clin Cancer Res,* 22(18):4567-73.
49. Infante J, Dees E, Olszanski A, Dhuria S, Sen S, Cameron S, Cohen R (2014). Phase I dose-escalation study of LCL161, an oral inhibitor of apoptosis proteins inhibitor, in patients with advanced solid tumors. *J Clin Oncol,* 32(28):3103-10.
50. Sas-Chen A, Aure M, Leibovich L, et al (2016). LIMT is a novel metastasis inhibiting lncRNA suppressed by EGF and downregulated in aggressive breast cancer. *EMBO Mol Med,* 8(9):1052-64.
51. Kim E, Ilagan J, Liang Y, et al (2015). SRSF2 Mutations Contribute to Myelodysplasia by Mutant-Specific Effects on Exon Recognition. *Cancer Cell,* 27(5):617-30.
52. Chang J, Yang D, Chang W, et al (2011). Small molecule amiloride modulates oncogenic RNA alternative splicing to devitalize human cancer cells. *PLoS One,* 6(6):e18643.
53. Kaida D, Motoyoshi H, Tashiro E, et al (2007). Spliceostatin A targets SF3b and inhibits both splicing and nuclear retention of pre-mRNA. *Nat Chem Biol,* 3(9):576-83.
54. Pham D, Koide K (2016). Discoveries, target identifications, and biological applications of natural products that inhibit splicing factor 3B subunit 1. *Natural Product Reports,* 33:637-47.
55. McClorey G, Wood M (2015). An overview of the clinical application of antisense oligonucleotides for RNA-targeting therapies. *Curr Opin Pharmacol,* 24:52-8.
56. Castanotto D, Stein C (2014). Antisense oligonucleotides in cancer. *Curr Opin Oncol,* 26(6):584-9.
57. Hua Y, Vickers T, Okunola H, Bennett C, Krainer A (2008). Antisense masking of an hnRNP A1/A2 intronic splicing silencer corrects SMN2 splicing in transgenic mice. *Am J Hum Genet,* 82(4):834-48.
58. Stevens M, Oltean S (2019). Modulation of the Apoptosis Gene Bcl-x Function Through Alternative Splicing. *Front Genet,* 10:804.
59. Dewaele M, Tabaglio T, Willekens K, et al (2016). Antisense oligonucleotide-mediated MDM4 exon 6 skipping impairs tumor growth. *J Clin Invest,* 126(1):68-84.