Recent Advances of Bcl-2 Inhibitors in Cancer Treatment

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Abstract: The Bcl-2 protein family plays an important role in regulating cell apoptosis, including proteins that inhibit apoptosis and promote apoptosis. In the process of tumorigenesis, the expression of anti-apoptotic proteins increases, and the expression of pro-apoptotic proteins decreases. The overexpression of anti-apoptotic Bcl-2 family proteins is closely related to the pathology and drug resistance of various hematopoietic malignancies and solid tumours. The overexpression of Bcl-2 protein provides conditions for the long-term survival of tumour cells, which can enhance the resistance of mutant cells to gene damage, so that abnormal cells with DNA damage cannot be identified and eliminated, and programmed cell apoptosis is inhibited. This leads to the occurrence of tumours. In the treatment of tumours, this protein site can be used as a target for targeted tumours treatment to achieve the effect of strong specificity and less adverse effects. The research on Bcl-2 inhibitor drugs has made great progress in recent years. This article reviews the application of Bcl-2 inhibitors and the drugs in targeted anti-tumour therapy in recent years at the gene and protein level, explores the advantages and disadvantages of this therapy and provides the basis for better targeted anti-tumour therapy in the future.

1. Introduction

The gene expression product of Bcl-2 gene belongs to Bcl-2 protein family which includes both antiapoptotic and proapoptotic proteins. Based on the structural and functional characteristics of the subunits, Bcl-2 protein family can be further divided into three subgroups which are subfamily 1, 2, and 3. The subfamily 1 of the Bcl-2 proteins is composed of the protein products of Bcl-2, Bcl-xl, Bcl-w, Mcl-1 genes whose structures consist of the homologous regions of segment BH1-4 comparing to the protein expressed by Bcl-2 gene. The proteins in the subfamily 2 have homologous regions of BH1-3, and these proteins are multidomain proapoptotic proteins. The proteins in the subfamily 3 belong to BH-3 only category, and they are proapoptotic in nature.

As is shown in Table 1, proteins in the same subfamily have the same structural domains which equip them with similar function. To be more specific, the proteins in the Bcl-2 protein family all include BH conserved gene sequences. In the genetic level, if the inhibitors bind specifically with the target mutated genes, the genes' transcription and further on translation will be blocked. For instance, the antisense oligonucleotide inhibitors of the Bcl-2 proteins exert their role through this mechanism achieving antitumour property by inhibition of antiapoptotic effect. While if the inhibitors act on specific regions in the Bcl-2 proteins through interacting with conserved BH regions, the binding of apoptotic proteins with Bcl-2 proteins will be prohibited. In this way antiapoptotic property of the members in Bcl-2 proteins' family can also be restricted, achieving the antitumour property. Drugs such as BH3 mimetic small molecule inhibitors functionalized through this pattern [1].

Table 1. Members of Bcl-2 proteins' family

		Structural domain					Forecasting
antiapoptotic		BH4	BH3	BH1	BH2	TM	Bcl-2, Bcl-xL, Bcl-w, Mcl-1
Proapoptotic	Multidomain		BH3	BH1	BH2	TM	Bax, Bak
	BH3-only		BH3			TM	Bik, Blk, Hrk, Bim, Bnip3
			BH3				Bid, Bad

2. Antisense oligonucleotide (ASO) inhibitors of Bcl-2 proteins

2.1 Mechanism of Action

ASO is one of the inhibitors for Bcl-2 proteins which is a short gene sequence and can specifically bound with target genes through complimentary base pairing to inhibit the expression of Bcl-2 genes to prevent the synthesis of Bcl-2 proteins. This oligonucleotide sequence is chemically synthesized and it usually acts on messenger RNA of Bcl-2 proteins to modulate RNA function. The mechanism is shown in Figure 1 [2]. To be more specific, ASO's mechanism can be further categorized into placeholder-only mechanism and RNA degradation mechanism. In the placeholder-only mechanism, the ASO binds to the target mRNA at specified site to inhibit the binding with anticodon sequence on the ribosome. In this way synthesis of Bcl-2 protein is blocked. In the RNA degradation mechanism, after ASO binding towards target mRNA at certain site, the endonuclease will be activated to decompose the complex of the ASO and target mRNA. Then the protein synthesis of antiapoptotic Bcl-2 proteins is also limited.

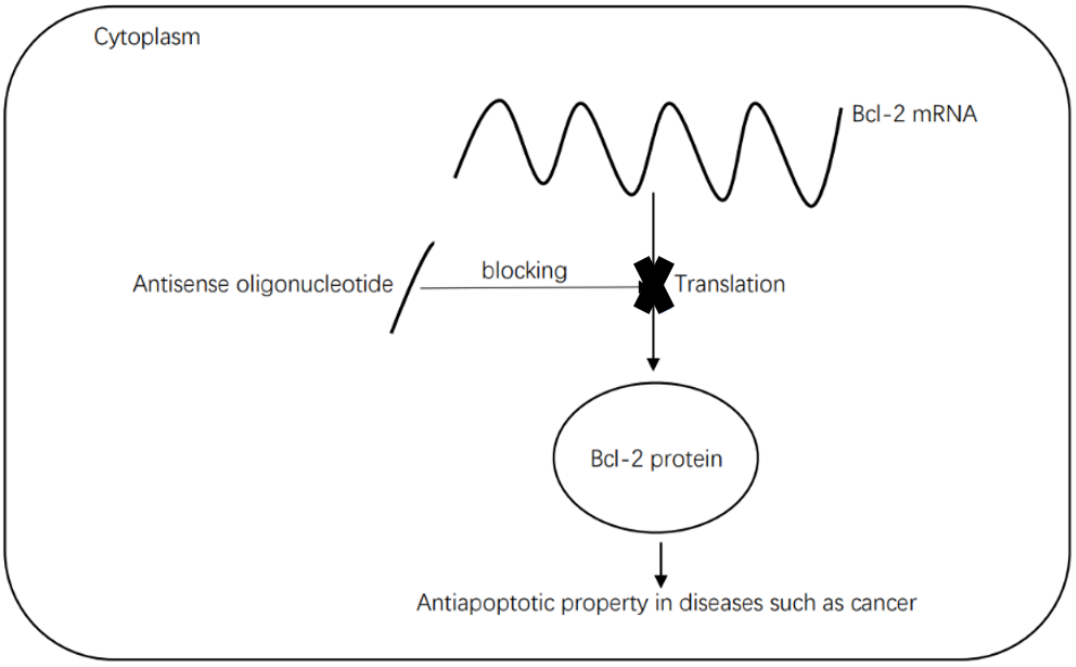

Figure 1. Schematic diagram showing the mechanism of antisense oligonucleotide Bcl-2 protein inhibitors [2].

2.1.1 SPC2996 (Beclanorsen)

SPC2996 is oligonucleotide molecule composed of 16 subunits, in which 4 DNA molecules is substituted with locked nucleotides. This is an ASO inhibitor targeting Bcl-2 mRNA, and it can be used to treat patients with chronic B lymphocyte leukemia (CLL). The Bcl-2 antiapoptotic protein in B lymphocytes is overexpressed in these patients, which would induce drug resistance towards chemotherapy, the aggressive progression of tumour and patients' poor survival. In the research carried out by Du¨rig et al., a novel ASO SPC2996 is used in Phase I/II clinical trial of CLL. In the preclinical trial stage SPC2996 performed effectively in cancer treatment with potent inhibiting

overexpression of antiapoptotic protein Bcl-2. In comparison with traditional Sulphur-substituted oligonucleotides, SPC2996 has higher affinity with targeting sequence, higher specificity and biological stability. Meanwhile, it has smaller immune irritant side effect [3].

2.1.2 Oblimersen

Another commonly used ASO inhibitor is oblimersen which has optimized therapeutic effect in the treatment of the drug-resistant melanoma whose drug resistant could be activated by the overexpression of Bcl-2 gene. Oblimersen is Sulphur-substituted ASO consisted of 18 bases which is distinctive comparing to SPC2996 with difference in 3 nucleotides. It can complimentarily bind with the sixth reading frame in Bcl-2 mRNA exon， prohibiting gene translation and further block synthesis of Bcl-2 protein to exert its antitumor property [4]. In addition, oblimersen can be applied in the treatment of extensive stage-small cell lung cancer (ES-SCLC) where cell division of tumour cells are fast with antiapoptotic protein Bcl-2 being overexpressed. Based on this circumstance, Charles et al. have analyzed the use of Oblimersen (G3139) which is the first Bcl-2 protein inhibitor with Sulphur substituted chain in place of phosphodiester bond in natural DNA [5].

2.2 Application and related problems

A great number of clinical researches have verified that ASO inhibitors have relatively high specificity both in vitro and in vivo, permitting them to be applied from in vitro experiments to preclinical animal trials and clinical trials for human beings. The design of this type of drugs is convenient, allowing them to be more easily formulated into commonly used intravenous dosage. This makes ASO more possible to be used in clinical treatment. In the clinical application or clinical trials, the ASO drugs have some limitations such as vulnerability to be hydrolyzed by nucleases, poorly permeable across cell membrane and the cost is comparatively expensive to synthesize this kind of drugs generating expensive cost for long-time treatment. In the clinical research, for the single use of oblimersen, although the tumour inhibition rate can reach 83% there is no significant difference of the Bcl-2 protein amount inside patients' blood. This might be generated from the inhibition of anticancer drug intake into tumour cells because of the occurrence of tumour that limits anticancer potency.

ASO has satisfying targeting inhibitory property towards mutated genes. At the same time, they are negatively charged with high water solubility which is critical for the drug in vivo potency. However, high water solubility makes this drug poorly permeable across biological barrier which is composed of phospholipid bilayer. Therefore, some biocompatible drug carriers can be used to transport drug molecules intracellularly. Also, because this drug belongs to nucleotides, they are prone to be hydrolyzed by the nucleases and be ineffective. Then to achieve stable delivery, the carriers such as liposomes can be applied to convey oblimersen into target cells [6].

3. Small-molecule inhibitor

Small-molecule inhibitor is a substance imitating the chemical and physical properties of small-cluster critical residues on the protein-protein interface. Conventional drug design focuses on inhibiting a single protein, which is generally the enzyme or receptor, because these proteins often contain a clearly defined ligand binding site, and small molecule drugs can interact with this site. The Bcl-2 small-molecule inhibitor plays a decisive role in the anti-apoptosis function of the associative hydrophobic groove within the BH3 domain of Bcl-2. By using small-molecule inhibitor to block this hydrophobic slit, it can effectively prevent dimerization after Bcl-2 binds with the downstream Bax or Bak, so as to remove the apoptosis inhibition function of Bcl-2 and make cells inclined to apoptosis.

3.1 ABT-737

3.1.1 Mechanism of Action

ABT-737 is a small-molecule inhibitor imitating the BH3-only function. As the antagonist, ABT-737 shows high affinity with the hydrophobic grooves of Bcl-xL and Bcl-2, which can damage the

Bcl-2/Bax association and cause apoptosis [7]. Previous studies show that ABT-737 can effectively inhibit the proliferation of HeLa cells, and its inhibition pathway of HeLa cell proliferation mainly consists of two aspects. On the one hand, ABT-737 can participate into the apoptosis signal pathway by inhibiting the Bcl-2/Bcl-xL function, which can effectively prevent dimerization after Bcl-2 binds with the downstream Bax or Bak, so as to remove the apoptosis inhibition function of Bcl-2. on the other hand, ABT-737 can activate the JNK signal pathway of cervical cancer to further activate c-Jun, so as to regulate the expression of Bim [8].

3.1.2 Application and related problems

As a single drug, ABT-737 can bind with other cell toxicants to treat acute myeloid leukemia (AML), multiple myeloma, lymphoma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL) and small cell lung cancer (SCLC) [8]. At present, ABT-737 is at phase I/II of clinical test. Previous experiments prove that ABT-737 can effectively kill primary CLL cells. Primary CLL cells are sensitive to ABT-737, which vindicates the use of primary CLL cells as BCL2 dependent human cancer model. Compared to BCL2 in the CLL cell, the content of MCL1 in complete or divided form is relatively low, which does not play an important role in determining reactions toward ABT-737 [9].

3.2 ABT-263

3.2.1 Mechanism of Action

ABT-263 is a small-molecule inhibitor of Bcl-2 family developed during the clinical development of ABT-737, and it has very similar structure and principle to its predecessor ABT-737. After using ABT-263, ABT-263 combines with the BH3 binding groove from BCL-2 protein family in the cytoplasm, which promotes the translocation of pro-apoptotic BH3-only protein BIM from BCL-2. Then, BIM is released, which triggers the mitochondria to release small hemoprotein, i.e., cytochrome c, and it leads to apoptosis [10]. Because ABT-737 has poor efficiency of oral administration, ABT-263 is improved on this aspect. In order to improve the oral administration effects of ABT-263, balance should be sought among the three aspects of target affinity, cell efficiency and oral absorption (see Figure 2).

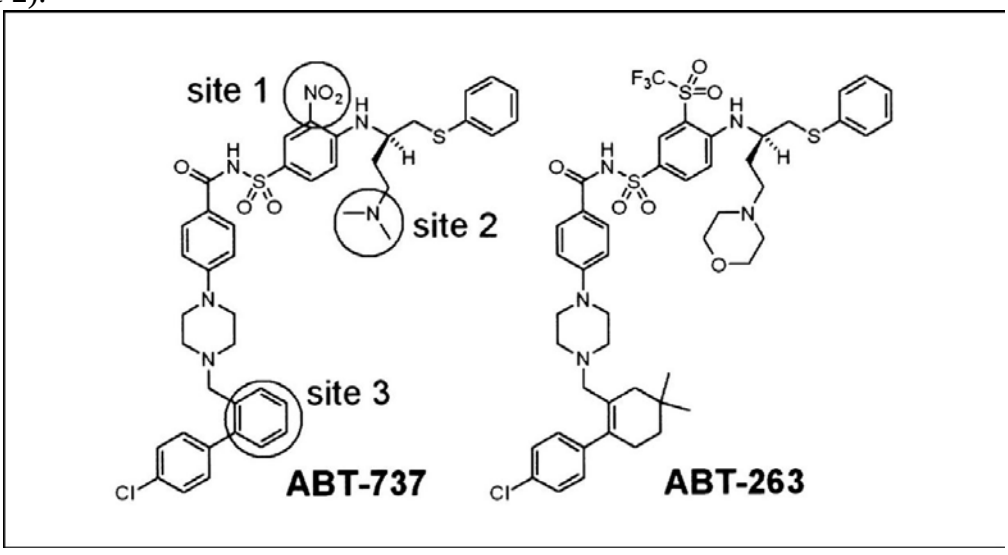

Figure 2. Comparison between chemical structures of ABT-737 and ABT-263 [11].

3.2.2 Application and related problems

ABT-263 is a second-generation product which can be taken orally developed on the basis of ABT-737. ABT-263 can enhance the radiotherapy and chemotherapy effects in treatment of small cell lung cancer (SCLC), follicular lymphoma and aleukemic leukemia. At present, ABT-263 is at phase I~II clinical test. Navitoclax (ABT-263) can inhibit BCL-XL, while BCL-XL is a key factor deciding

normal survival and death of blood platelets, so it can cause apoptosis of platelets and trigger acute thrombocytopenia, and this process will occur 48~72h after taking the drug. However, during the research and development process, Navitoclax achieved certain effects in relapsed or refractory CLL, the partial response (PR) was around 35%, and the median progression-free survival (PFS) was approximately 25 months [12]. If ABT-263 can have specific binding with Bcl-2 while does not react with BCL-XL, it can reduce the apoptosis rate of blood platelets and better improve the safety of drugs.

3.3 ABT-199

3.3.1 Mechanism of Action

ABT- 199 is a BCL-2 specific BH3 domain mimic derived from ABT-737, and as a third-generation product from the ABT drug family, it shows strong pro-apoptotic function in treatment of hematological tumor cells. ABT-199 (Venetoclax) is an oral, selective and strong BCL-2 inhibitor, which is independent from TP53. When ABT-199 is used as single treatment for chronic lymphocytic leukemia (CLL) patients with severe pretreatment, including patients with poor features such as deletion of chromosome 17p, it can induce a high response rate and great response quality. Venetoclax can bind with CD20 antibody rituximab. CD20 antibody rituximab is an established component of CLL treatment, and it is found that such combination can overcome drug resistance to Venetoclax induced by the microenvironment.

3.3.2 Application and related problems

ABT-199 (as shown in Figure 3.) presents inhibition of various tumors, such as leukemia, non-Hodgkin's lymphoma (NHL) and multiple myeloma. In clinical tests (M12-175, II M13-982 and M14-032), 350 patients with relapsed or refractory CLL participated in the experiment, and the most common adverse reactions included diarrhea, neutropenia, nausea, anemia, fatigue and upper respiratory infection. As for the effects of drug, the first clinical test of ABT-199 was carried out on patients with relapsed/refractory CLL or NHL. The 105 patients participating in the experiment showed great tolerance of ABT-199, with occasional adverse reactions mentioned above. The objective remission rate (ORR) was around 77%, the complete remission (CR) was 23%, and the partial remission (PR) was 54%. It is the only small-molecule inhibitor in the ABT drug approved by the FDA and is successfully launched in the market.

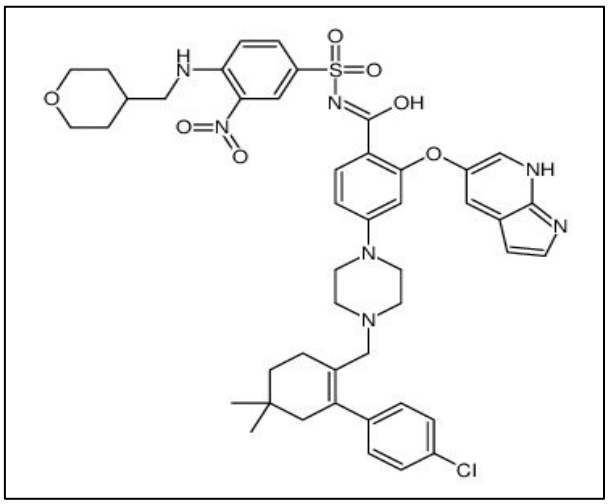

Figure 3. Chemical structure of ABT-199.

3.4 Gossypol and its derivatives

3.4.1 AT-101 (Gossypol)

The structure in Figure 4a is AT-101 (gossypol)’s chemical structure. AT-101 is an effective anticancer drug in preclinical trial which can inhibit many types of tumours with in vitro anti-

multiplication potency and in vivo anticancer property. This is a naturally generated small molecular BH3 mimetics. Serving as Bcl-2 protein inhibitor, gossypol has good binding affinity with antiapoptotic proteins Bcl-2 (Ki=320 nM), Bcl-xL (Ki=480 nM) and Mcl-1 (Ki=180 nM) at BH3 binding site [13].This drug mimics BH3-only proteins, and it can bind to proapoptotic proteins such as Bak on the outer membrane of mitochondria facilitating proapoptotic protein to form oligomer state, activating Bak to induce tumour cells' apoptosis and improve the release of cytochrome C with the damage of mitochondrial membrane (Figure 4b). Gossypol has both in vitro and in vivo potency in limiting multiplication of many types of tumour cells, such as squamous cell carcinoma of the head and neck, glioblastoma, prostatic cancer, breast cancer and lung cancer. The biological activity of gossypol is characterized by -CHO and 6-OH that guarantee AT-101's optimized solubility.

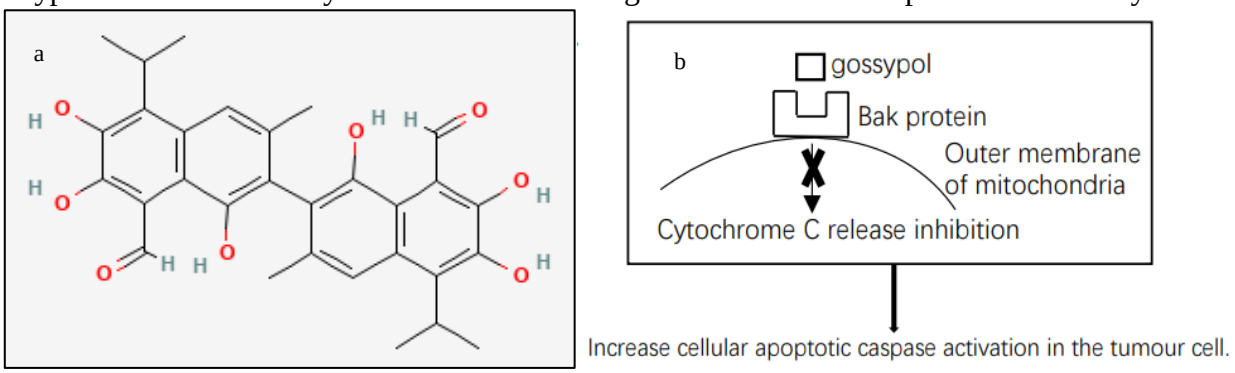

Figure 4. (a) Chemical structure of AT-101; (b) Anticancer mechanism of AT-101.

Currently, AT-101 has achieved good therapeutic potency in the treatment of glioblastoma. Meanwhile, when gossypol and polyselenium paclitaxel are in combined therapy, the overall therapeutic potency would reach 81.82%. Comparing to 65.22% effectiveness for the single use of the polyselenium paclitaxel in treatment, the combined therapy is statistically significant in improving lung cancer treatment. Apart from this if AT-101 can also be used with paclitaxel and carboplatin in treating solid tumours such as esophagus cancer. When combinationally used together with polyselenium paclitaxel, AT-101 can be applied to treat recurrent, locally advanced or metastatic head and neck cancer. When AT-101 is in combination with cisplatin and etoposide, this can serve as the therapy in ES-SCLC which achieve complete or partial disease remission [14].

AT-101 can be well-sourced, cheap, highly clinically safe and less toxic. It can be used in combination with radiotherapy or chemotherapy. Comparing to other types of Bcl-2 inhibitors like ASO or peptide inhibitors, gossypol has significant advantage at bioavailability aspect, making it as potential candidate in anticancer treatment.

However, gossypol has some adverse effects like possibility to induce hypokalemia. Although this circumstance is rare, in drug administration it should be concerned. In addition, like other drugs, resistance might occur if gossypol is extensively used so that long time administration of gossypol should be avoided. Therefore, in later application of gossypol, researchers tried to chemically modify the structure at hydroxyl and aldehyde groups. Some advances have been achieved for instance team of Ilkevych have condensation reaction between hydrazine compounds and carbonyl group in aldehyde group to synthesize hydrazone compounds. Verified through DPPH free radical clearance assay, the hydrazone compounds have higher anticancer bioactivity about 5 times that of gossypol and smaller toxicity.

3.4.2 Apo gossypol

Apogossypol (Figure 5.) is a new type of small molecule inhibitor of Bcl-2 family proteins, which has been proven to have anti-tumour activity. Its structure is based on gossypol, with two aldehyde groups removed, and Bcl-2 family proteins have certain in vitro anti-apoptotic activity. The apogossypol synthesized by removing two aldehyde groups maintains the anti-cancer effect and showing lower activity [15, 16].

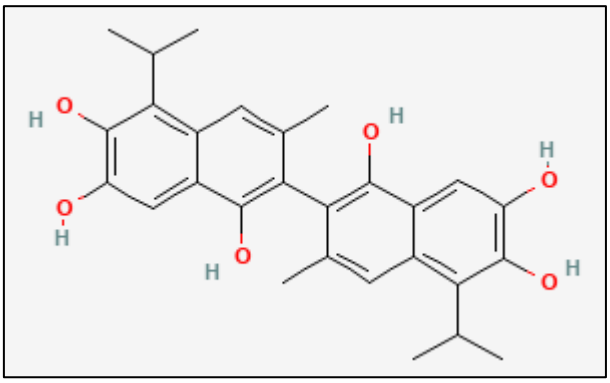

Figure 5. Apogossypol structural formula.

Apogossypol competitively binds to the BH3 domain of Bcl-xl, leading to the release of pro-apoptotic proteins Bim and Bax, and ultimately causing apoptosis [17].

Subsequent studies on the mechanism of apogossypol have shown that the reorganization of the endoplasmic reticulum (ER) membrane mediated by apogossypol can prevent BH3 mimic-mediated apoptosis [18]. This is an unexpected result. So, the research on the mechanism of Apogossypol is still going on. Apogossypol has good anti-proliferative activity in nasopharyngeal carcinoma, prostate cancer, human leukemia mononuclear lymphoma, diffuse large cell lymphoma, follicular lymphoma, pancreatic cancer cells and human hepatocellular carcinoma, and it has small toxic [19, 20]. And the synthetic derivative apogossypolone can effectively inhibit the growth and proliferation of gastric cancer and prostate cancer cells in vivo and in vitro [21, 22].

In prostate cancer, apogossypol has obvious anti-tumour activity in vitro and in vivo. Apogossypol can bind to Bcl-2 family proteins, prevent pro-apoptotic proteins from binding to the BH3 domain, and release pro-apoptotic proteins to induce apoptosis [23]. Apogossypol may be a promising new therapy for prostate cancer. As a gossypol derivative, apogossypol shares same active anticancer structural region with AT-101. Through binding with BH3 structural region, both of these compounds can inhibit activity of antiapoptotic Bcl-2 proteins. The difference is that in apogossypol bioactive aldehyde groups have been eliminated. After orally administration, gossypol and apogossypol act as competitive antagonists of Bcl-2 protein to compete binding site of BH3 peptide with proteins like Bcl-2, Bcl-xL, Mcl-1, Bcl-W, Bcl-B in Bcl-2 protein family. based on this aspect, both of these compounds can be wide-spectrum antagonist of Bcl-2 proteins to treat two or more malignant tumours with Bcl-2 proteins overexpressed.

At current stage gossypol has been applied to treat malignant tumours such as solid tumours, lymphomas, leukemia in Phase I/II clinical trials. Although this drug has certain antitumour activity, it occupies certain hepatotoxicity and gastrointestinal toxicity. Based on analysis performed by University of California San Diego on patients with CLL after AT-101 administration, the toxicity is associated with the doses.

Because of weak binding affinity and fast plasma clearance, the half-life of gossypol in blood is only 2.8 hours, then the dose administered is comparatively high which makes the toxicity more likely to be generated. However, after elimination of aldehyde groups in gossypol, apogossypol's inhibitory potency towards Bcl-2 protein is not significantly weakened. In the meantime, comparing to gossypol's blood clearance, the variation of apogossypol's blood concentration changes quite slowly with time, therefore the dose administered for apogossypol can be effectively reduced, avoiding occurrence of the significant toxicity. Comparing to gossypol, apogossypol might have better clinical application and potency. However, the clinical anticancer activity is not so clear because research of gossypol and its derivatives is at clinical trials [24]. However, because apogossypol has poor absorption and permeability, and it is not quite stable [25], its possibility in clinical application is limited. As a consequence, some people continue to reform on its basis and produce a new small molecule inhibitor Ch282-5 with a good effect on inhibiting colon cancer cells, through which its toxicity is reduced while its hydrophilicity is increased [26].

3.5 GX15-070 (Obatoclax mesylate)

3.5.1 Mechanism of Action

As is shown in Figure 6., GX15-070 or obatoclax mesylate is a small molecular BH3 mimetic Bcl-2 protein inhibitor. GX15-070 presents binding affinity towards antiapoptotic Bcl-2 proteins such as Bcl-2, Bcl-xL, Bcl-w and Mcl-1 in Bcl-2 protein family. Obatoclax mesylate can bound specifically with homologous BH3 region, inhibiting these antiapoptotic factors binding with proapoptotic proteins for instance Bax and Bak through BH3 region. Further on the apoptosis of tumour cells is activated achieving antitumor effect. This drug can be used synergistically with other anticancer drugs, increasing cytotoxic potency towards tumour cells.

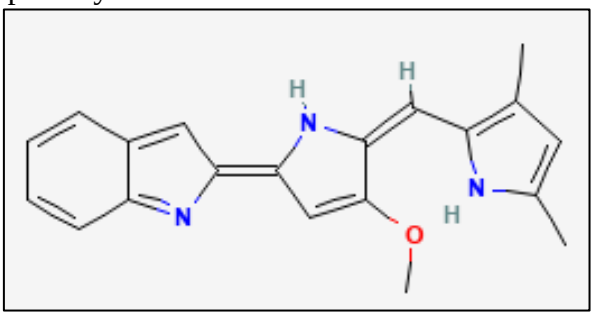

Figure 6. Chemical structure of obatoclax mesylate.

3.5.2 Application and related problems

GX15-070 can be applied into the treatment of small cell lung cancer which is a type of lung cancer with high malignant degree. The current treatment is commonly the combined application of platinum with other anticancer drugs. Obatoclax mesylate has been applied in Phase I clinical trial of small cell lung cancer and it has increased the patients' survival rate to a certain degree [27]. However, GX15-070 has comparatively weaker inhibition potency on small cell lung cancer comparing to inhibition therapy targeting PARP protein [28]. At the same time, this drug is likely to generate toxicity adverse effect in central nervous system, which might limit its use in clinical application [27].

3.6 BDA-366

3.6.1 Mechanism of Action

In recent years, Bcl-2 inhibitors that mimic BH3 domains to induce apoptosis have been tested in human clinical trials, but the clinical efficacy is limited. Therefore, targeting the BH4 domain of Bcl-2 has become a new means of inducing cell apoptosis and anti-tumor. The BH4 domain is necessary for the survival activity of Bcl-2, and removal of this domain can transform Bcl-2 from survival to a killer molecule [29].

A new small molecule inhibitor BDA-366 selectively targets the Bcl-2 BH4 domain resulting in changes in the Bcl-2 conformation and exposing the BH3 death domain. This conformational change activates the activity of Bax in cell-free systems and tumor tissues [30]. Therefore, BDA-366 is considered to be related to the expression level of Bcl-2. However, subsequent experiments have shown that the sensitivity of apoptosis to BDA-366 has nothing to do with the level of Bcl-2 protein in the corresponding cancer cells [31]. BDA-366 can neither directly activate the formation of Bax pores nor convert anti-apoptotic Bcl-2 into pro-apoptotic proteins. In addition, BDA-366 has no effect on intracellular Ca2+ homeostasis of cancer cells. Finally, BDA-366 seems to promote the down-regulation of Bcl-1 and the dephosphorylation of Bcl-2 without affecting the levels of Bcl-2 and Bcl-XL proteins.

3.6.2 Application and related problems

The mechanism of action of BDA-366 may be related to the structure of anthracyclines [31]. Such drugs have been used clinically for decades to treat cancers such as leukemia and lymphoma [32]. The main mode of action of these drugs is the inhibition of topoisomerase II, the insertion of DNA

and the generation of reactive oxygen species. In addition, anthracycline compounds can cause the down-regulation of mcl-1, thereby inhibiting non-selectively with Bcl-2/Bcl-xl The agent ABT-263 synergistically kills cancer cells [33]. Therefore, the mechanism of action of BDA-366 may have multiple ways to cause cell damage and apoptosis.

BDA-366 shows potent antitumor activity in lung cancer xenografts. It can effectively inhibit the growth of lung cancer and a certain dose is effective and safe for mouse lung cancer models [30]. Multiple myeloma (MM) is a heterogeneous plasma cell malignant tumor. BDA-366 can induce apoptosis of MM cells and inhibit the growth of MM cells in vitro [34].

The specific BH4 domain targeting Bcl-2 is a new tumor treatment strategy. Compared with the traditional BH3 domain targeting, this is a relatively new attempt. Importantly, not all members of the Bcl-2 family share the BH4 domain. Therefore, it has not been used in clinical practice.

3.7 TW-37

3.7.1 Mechanism of Action

TW-37 is a small molecule inhibitor of Bcl2 family proteins, which can induce the anti-cancer mechanism of a variety of cancers. TW-37 binds to the Bcl-2 homeodomain 3 (BH3) to prevent the pro-apoptotic protein from combining with Bcl-2, thereby inducing apoptosis. TW-37 is a second-generation benzene sulfonic acid derivative of gossypol isolated from cotton seeds and roots. Its purpose is to bind BH3 in Bcl-2 with high affinity to make Bcl-2, Bcl-xl and Mcl-1 induces apoptosis [35, 36]. TW-37 can induce S-phase cell cycle arrest by regulating several important cell cycle-related genes such as p27, p57, E2F-1, cdc25A, CDK 4, cyclin A, cyclin D 1 and cyclin E [37, 38]. Other experiments have shown that in inhibiting the growth of pancreatic cancer cells, part of the anti-tumor activity of TW-37 is mediated by the inactivation of Notch-1 and NF-κB signaling pathways [38].

3.7.2 Application and related problems

Tw-37 is an effective inhibitor to inhibit the growth of endothelial cells and head and neck tumor cells [39]. TW-37 can improve the chemotherapy sensitivity of nasopharyngeal carcinoma and has acceptable toxicity to healthy tissues. Therefore, TW-37 is a promising adjuvant drug for nasopharyngeal cancer chemotherapy [40]. In addition, experiments have proved that in preclinical studies of neuroblastoma, TW-37 has strong single-dose cytotoxicity both in vitro and in vivo. Therefore, combined inhibition of Bcl-2/Mcl-1, such as the inhibitory effect of TW-37 on N-Myc amplified neuroblastoma, may be an effective treatment strategy [41].

On the other hand, TW-37 has anti-cancer activity on human oral cancer cell lines MC-3 and HSC-3. TW-37 shows obvious anti-cancer activity in human oral cancer cells by inducing apoptosis. But the experimental results found that MC-3 cells may develop resistance to TW-37 in the later stage [42]. Therefore, although BH3 mimics for Bcl2 are effective against cancer cell apoptosis, especially hematological malignancies, the treatment of single-drug BH3 mimics is still limited [43, 44]. Therefore, the combined drug therapy that needs to be done later is very necessary.

3.8 Disarib

3.8.1 Mechanism of Action

In 2016, a new type of Bcl2 specific inhibitor Disarib was discovered that mainly binds to the BH1 domain. It can effectively and selectively bind to different Bcl-2 high cancer cells for targeting, and retaining other members of the anti-apoptotic family [45]. Disarib can significantly reduce mitochondrial membrane potential (MMP) [45]. It causes cell death by activating the intrinsic pathway of cell apoptosis. Further experiments proved that Caspase 9, Caspase 3, PARP 1 and other endogenous pathway proteins were also activated. CYTOCHROME C and SMAC/DIABLO have also been released. And when Disarib is treated in a concentration-dependent manner, the activated Bak level is higher. The results suggest that Disarib treatment can induce the intrinsic pathway of cell apoptosis [46]. Subsequent research discovered a new mechanism of Disarib-mediated cell death. Disarib can specifically destroy the interaction of Bcl2-Bak on the outer mitochondrial membrane,

thereby inhibiting the Bcl2-Bax protein complex, thereby initiating the apoptotic pathway. In addition, Bak homodimers may also lead to the formation of mitochondrial membrane pores, leading to the release of cytochrome C, the formation of apoptotic bodies in the cytoplasm, the scission of Caspase 9 and 3, which ultimately leads to DNA rupture and cell death [47].

3.8.2 Prospective

Disarib has higher efficacy with other Bcl2 inhibitors such as gossypol, HA-14, ABT-199[45]. Compared with ABT-199, Disarib has better cytotoxic potential, which may be because Disarib has better application in clinical trials as a single drug tool against Bcl2 high cancer [46]. In addition, compared with gossypol, HA-1 and other Bcl2 inhibitors, Disarib caused a significant increase in tumor cell death [45]. Disarib has proved that it is safe as a small molecule inhibitor [48]. A study performed Disarib's pharmacokinetics, pharmacodynamics and acute toxicity analysis on rodent-Wistar rats shown that Disarib does not cause any toxicity when used in rats [49].

On the other hand, after treating Disarib with paclitaxel, it was found that Disarib was able to cause a pronounced cytotoxic effect compared to the use of each compound alone [46]. It has also been reported that paclitaxel takes Bcl2 as the center and binds to the loop region of Bcl2, leading to the activation of apoptosis [50]. Therefore, the combined effect of Disarib and paclitaxel may be an effective strategy to eliminate the Bcl2 function of cancer cells. Many researches on Disarib principles and toxicity trials from 2016 to the present have provided the basis for further preclinical and clinical trials.

4. Conclusions and prospects

Bcl-2 targeted inhibitors can induce apoptosis in tumor cells with high Bcl-2 expression and make these cells more sensitive to conventional chemotherapy. Early small molecule inhibitors targeting Bcl-2, such as ABT-737 and levo-gossypol (AT-101), have shown poor clinical efficacy. With the development of research, many new inhibitors targeting Bcl-2 have been developed on the basis of these early drugs, and their efficacy has been improved, adverse reactions have been reduced, and the clinical application prospect is good. However, due to the different pathogenesis of various tumor cells, the Bcl-2 protein family inhibitors have only achieved great success in the treatment of hemolymph system tumors. Monotherapy or combination therapy for Bcl-2 family inhibitors in solid tumors such as breast cancer, non-small cell lung cancer and head and neck cancer is still under study. Due to the obvious heterogeneity of tumor cells, various drugs still cannot solve the problem of drug resistance in tumor treatment. In summary, the study of Bcl-2 inhibitors has made breakthrough progress and provided new ideas for the treatment of malignant tumors. Further research is needed on how to overcome drug resistance.

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