The Application of BH-3 Only Mimetics in Tumor Therapy

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Abstract: Tumors are divided into benign and malignant, and the further development of malignant tumors becomes cancer. In recent years, the number of deaths from cancer worldwide increasing rapidly. To better fight cancer, targeted therapy is a more precise method. Among them, BH-3 only protein is a representative protein for targeted therapy. BH-3 only protein is a BCL-2 family protein with only one BH domain and acts as a pro-apoptotic protein to regulate cell apoptosis. Different BH-3 only proteins have different binding options with anti-apoptotic proteins in the BCL-2 family. BH-3 only protein has an excellent performance in inhibiting cancer cells, and the research of BH-3 only mimics constantly updated. This paper will introduce drug research on tumor treatment by studying the effects of targeted BH-3only mimics and expanding drugs' development for its related family proteins.

1 INTRODUCTION

Programmed cell death, or apoptosis, is a unique form of suicidal cell death accompanied by cell size reduction and chromatin concentration (Kroemer et al. 2005). Apoptosis controls the number of cells in an organism, eliminating harmful or virus-infected cells. There are mainly three pathways regulating apoptosis: mitochondria-mediated pathway, death receptor pathway, and endoplasmic network pathway (Kerr, Wyllie, Currie 1972). The interactions of the BCL-2 family control the mitochondrial apoptosis pathway (Levine, Sinha, Kroemer 2008). Structural feature similarity is determined by sequence homology. Hydrophobic slits are formed between the four (BH1-BH4) domains of protein-protein interaction, which are involved in the proapoptotic protein BH3 domain uptake through heterodimerization. BCL-2 anti-apoptotic proteins include BCL-2, BCL-W, BCL-XL, MCL-1, and A1 (BCL2A1/ BCL-1), containing four BH homologs have similar protein 3D structures (Figure 1a) (Shamas-Din, et al. 2011).

The balance of these interactions determines the life cycle length of the cells expressing the corresponding protein. PUMA, BIM, tBID can bind to all members (Ley, et al. 2005, Qian, Zhang, Zhi 2017). TBID can antagonize the pro-survival function of BCL-2 and can directly combine with BAX and BAK to initiate apoptosis (Merino, et al. 2009, Hutt 2015). Except for BH-3, only protein, which can bind to any pro-survival protein, the binding of other BH-3 only proteins are selective (Figure 1b). BH-3 only protein will be selected based on the above binding to respond to apoptosis signals so that these BCL-2 anti-apoptotic proteins are isolated from BAX and BAK (Singh, Letai, Sarosiek 2019).

As of 2018, among the 18 million cases of cancer globally, the number of deaths is estimated to have reached 9.6 million, and cancer has become the world's second prominent cause of death (Copur 2019). During the research process, the excellent therapeutic effect of targeted drug therapy emerged as a hot research object in cancer treatment worldwide. BH-3 mimics anti-tumor drugs are a new type of anti-tumor drugs targeting BCL-2 family anti-apoptotic member proteins. This article will introduce the clinical drug course and progress of BH-3only mimics developed by the selective binding of different BH-3 only proteins.

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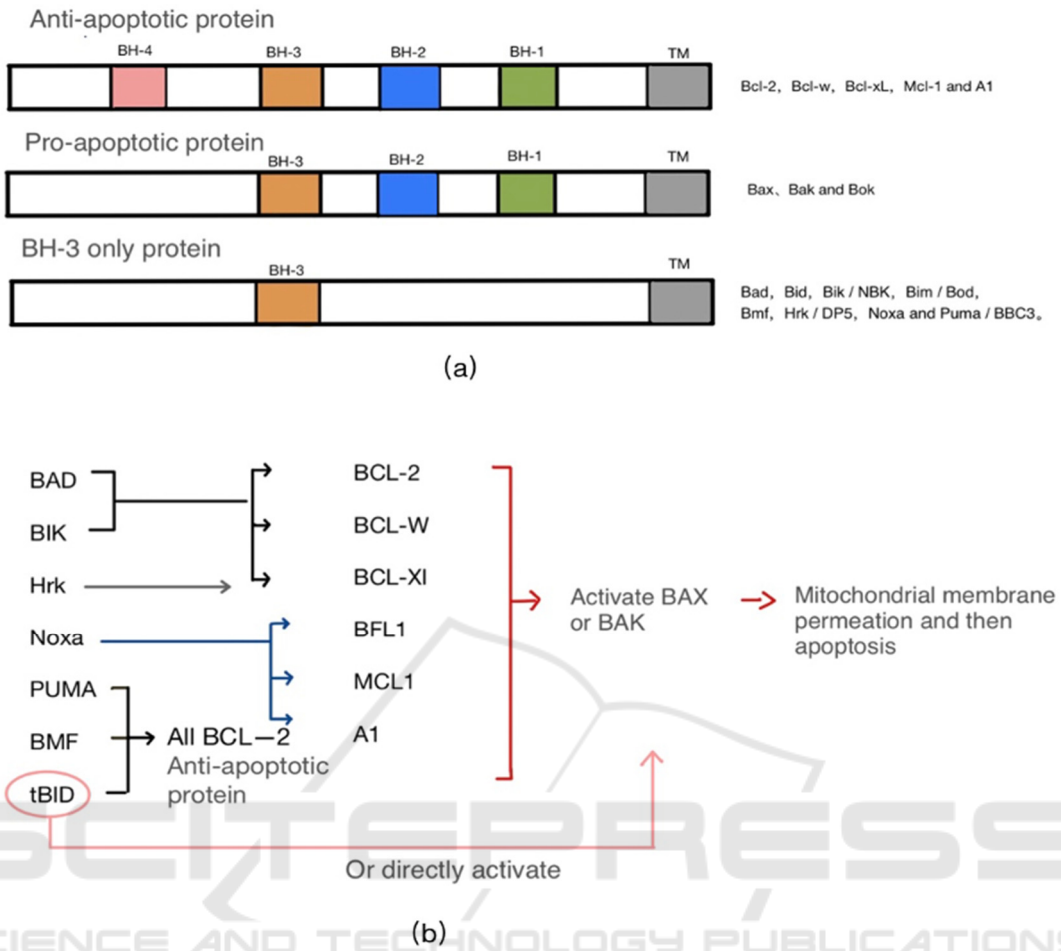

Figure 1: BCL-2 family protein structure and selective binding.

2 BH-3 ONLY MIMETICS

Cancer cells are different from normal cells in that they have three major characteristics: infinite proliferation, transformability, and easy metastasis. These characteristics bring great difficulties to the treatment of cancer. Since discovering the apoptotic properties and three-position structure of BCL-2 anti-apoptotic family proteins, research on its chemical resistance and protein inhibitors has continued. Targeted cancer treatment is the current mainstream idea of clinical treatment of cancer to improve the cure rate and the survival time of patients. Overexpression of anti-apoptotic BCL-2 family proteins can drive cancer cell proliferation or resistance to chemotherapy drugs. The combination of BH-3 only protein and the anti-apoptotic BCL-2 family protein leads to the release and activation of Bak and Bax, which leads to cell apoptosis. Therefore, BH3 mimics have great prospects in developing drugs targeting the anti-apoptotic BCL-2 protein (Baell, Huang 2002).

2.1 Target BCL-2 Protein2.2 Target MCL-1 Protein

2.1.1 S55746

Its selective characteristics indicate that it does not significantly bind to MCL-1, BFL-1 (BCL2A1/A1), and has a poor affinity for BCL-XL. S55746 can be taken orally and is not harmful to BCL-X1-dependent cells such as platelets. The combination of S55746 and ABT-199 is different. S55746 occupies the S1/2/3 region and forms a hydrogen bond with the carboxyl group of the A149 skeleton in the S2 residue (Casara, et al. 2018), forming a highly specific binding. ABT-199 occupies more protein surface area, including S2/3/4/5 (Souers, et al. 2013).

2.1.2 Phenothiazine

Tumor cells have developed a variety of strategies to achieve proliferative advantages. Researchers are developing chemicals to impede the interaction between the pro-apoptotic protein, thereby mimicking the mechanism of action between the pro-apoptotic protein and the BH-3 domain (Degterev, et al. 2001). Other drugs in the article, such as ABT-737 and ABT-263, have appeared and used for many years. To increase the treatment of tumors, the interaction between phenothiazine and biofilm is due to the amphiphilic nature of the molecule. The thiazide core is relatively hydrophobic (Philot, et al. 2016, Homem-de-Mello, Mennucci, Tomasi, et al. 2005). Therefore, phenothiazine drugs have a great potential for the anti-apoptotic protein BCL-2 inhibitory effect and appear in the actual treatment process as drugs for the treatment of tumors.

2.1.3 Gossypol /ApoG2

Gossypol is a polyphenol extracted from the Campagnaceae plant (Flack, et al. 1993). Gossypol is helpful in human clinical trials of stage ii cancer. Gossypol is a well-known toxic compound. However, Apogossypolone (ApoG2) can be obtained by removing two aldehyde groups (Arnold, et al., 2008). Moreover, in clinical trials, due to the existence of two reactive aldehyde groups, which are related to side effects of gossypol, such as vomiting and diarrhea, the design and synthesis of ApoG2 eliminates the reactive groups, minimizing side effects, and ApoG2 has higher stability and efficacy than its parent compound.

Follicular lymphoma (FL) is the fifth most diagnosed cancer (Rogers 2005). ApoG2 was found to be used in the treatment of follicular lymphoma. Lymphoma (FL is very effective, it inhibits cell growth by lysing the cell lymphoma cell line (WSU-FSCCL). The cell growth inhibition rate is 50%. In nasopharyngeal carcinoma cells, ApoG2 completely blocks the anti-apoptotic function of BCL-2 family proteins (McDermott, Dutt, Watkinson 2001). ApoG2 has three types. Researchers have shown through research that ApoG2 may be a new BCL -2 family protein inhibitor. By targeting these proteins, it may become a promising drug for the treatment of nasopharyngeal carcinoma.

2.2 Target MCL-1 Protein

2.2.1 S63845

As a BH-3 mimic, S63845 can bind to MCL1 with high affinity and specificity. S63845 can effectively kill MCL1-dependent cancer cells, although as a single drug, it can effectively act on multiple myeloma, leukemia, and lymphoma cells (Kotschy, et al. 2016). S63845 binds to MCL-1 more efficiently and specifically. The binding affinity of S63845 synthetic MCL-1 inhibitor for MCL-1 is 20 times that of A-1210477, and the effect of killing MCL1-dependent H929 multiple myeloma cells is 1000 times that of A-1210477 (Merino, et al. 2017, Li, Z., He, and Look 2019). The combination of S63845 and ABT-737, ABT-263, ABT-199, which have a low binding capacity to BCL-1, can improve the therapeutic efficacy (Merino, et al. 2017). Experiments have shown that the combined administration of S63845 and ABT-199 can more effectively induce human T-lymphocytic leukemia (T-ALL) cells (Li, Z., He, and Look 2019).

2.2.2 S64315/MIK665

S64315 is simulant S63845, and the effect is better than S68345 (Hird and Tron 2019). Compared with S63845, there is no clinical trial (Szlavik, et al. 2020). Acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) (NCT03672695 and NCT 02979366), and multiple myeloma (MM) (NCT02992483), the first phase of MDS and MM trials just ended in March 2021.

2.2.3 VU661013

VU661013 is a derivative of indole-2-carboxylic acid, which can reduce the expansion of AML cell lines (Ramsey, et al. 2018). VU661013 can also make ER+ breast cancer cells apoptosis and will not up-regulate BCL-2 or BCL-XL in ER+ breast cancer cells during treatment (Williams, et al. 2019), which is better than S68345.

2.3 Target BCL-XL Protein

2.3.1 A-1155463/A-1331852

BCL-XL is an anti-apoptotic protein located in mitochondria and one of the key factors of cell apoptosis. Malignant pleural mesothelioma (MPM) has been tested with a series of BH-3 mimics, and BCL-XL is the main pro-survival protein. Malignant pleural mesothelioma (MPM) is one of the cancers

with the lowest survival rate. This experiment discovers that BCL-XL is a feasible breakthrough in treating malignant pleural mesothelioma (MPM) (Arulananda, et al. 2020). A-1331852 is a potent and selective BCL-XL inhibitor that can be taken orally. Using structure-based drug design to redesign the BCL-XL inhibitor A-1155463 reported earlier is also a further discovery of BCL-XL inhibitors. The inhibitor is a small molecule (Wang, et al. 2020). Research on A-1155463 found that it has a huge effect on BCL-XL-dependent tumors, and it also retains the cell system of BCL-2 and MCL-1. The Epstein-Barr virus (EBV) related T cell and natural killer (NK) cell malignancies, A-1331852-induced apoptosis ENKTL cell line SNK6 established a xenograft model provides evidence that A-1331852 treatment may be effective It is beneficial in vivo (Arulananda, et al. 2020). After treatment with A-1331852, it can continue to induce apoptosis to achieve the therapeutic effect.

2.4 Target Multiple Proteins

2.4.1 ABT-737

ABT-737, as a kind of BH-3 only mimic, can bind to Bcl-2, BCL-W, and BCL-XL (Shin, et al. 2015). ABT-737 leads cancer cells to apoptotic, but it is harmful to common cells (Oltersdorf, et al. 2005). Although it just can be combined with the MCL-1 that involves many apoptosis pathways, ABT-737 still has excellent prospects in clinical. Overexpression of MCL-1 and A1 will weaken the sensitivity of cells to drugs. If MCL-1 is inactivated, overexpression of BCL-2 will not reduce the cytotoxic activity of ABT-737 for cancer cells, but overexpression of BCL-CL will relatively reduce the efficacy of the drug. Mcl-1 is an unstable protein, and the half-life of Mcl-1 mRNA and MCL-1 protein is very short (Anderson, et al. 2016). Seliciclib, cyclin-dependent kinase inhibitor and protein synthesis inhibitor cyclohexylamine (CHX), can reduce MCL-1 levels and significantly increase the sensitivity of cells to ABT-737 (F.van 2006). This report shows that the combination of inactivated MCL-1 and ABT-737 is promising for clinical treatment. It has been reported that ABT-737 is particularly sensitive to acute myeloid leukemia stem cells. Phenformin can increase cell sensitivity more on this basis, and ABT-737 combined with phenformin can be more suitable for targeting hematological malignancies (Velez, et al. 2016).

2.4.2 ABT-263

ABT-737 is the most successful and potential BH3 mimic compound developed by Abbott. In recent years, this mimic has played an indispensable role in regulating apoptosis therapy. However, ABT-737 also has restrictions. That is, it cannot be taken orally. The full name of ABT-263 is navitoclax (Tse, et al. 2008). But it is different from ABT-737. For chemotherapeutics that use drugs to adjust cell apoptosis, cell apoptosis resistance caused by inducing factors is a key hindrance to cancer control. To study this problem, a phase I experiment was conducted in 2007 (Gandhi, et al. 2011). These problems reflect the need for improvement of ABT-263. In the records of Gernot et al. in 2015, wogonin, apigenin, chrysin, etc., can reduce the efficacy of enhancing ABT-263 and thus reduce the dosage of drugs (Polier, et al. 2015). In an experiment on ABT-263 in 2020, ABT-263 affected bone changes and cell damage to a certain degree in aged mice (Sharma, et al. 2020). The experiment is based on the in vitro oral administration of several groups of old mice. The use of isolation and contrast culture of bone marrow stromal cells from ABT-263 or carrier-treated mice obtained experimental results.

2.4.3 ABT-199

Following ABT-737 and ABT-263, there have been new advances in BCL-2 inhibitors. ABT-199 is a new type of small molecule inhibitor. Although the first two have targeted treatment characteristics, the selectivity of ABT-199 is special. The treatment of tumors by BH-3 mimics is achieved by adding drugs to the metabolic mechanism. ABT-199 can resist tumors and reduce the damage to certain cells during treatment (Davids and Letai 2013). As mentioned above, ABT-263, the inhibitor, can be taken orally and cause damage to platelets. Souers and colleagues redesigned reverse engineering in which ABT-199 selectively killed BCL-2 cells without destroying BCL-XL cells. Proved that ABT-199 is a highly effective and selective inhibitor. In other developments, ABT-199 has also made new breakthroughs (Jakubowska, et al. 2019). It will not destroy the steady state of certain ions in the internal environment. ABT-199 has a slight effect on the content of ions in the solute and does not significantly change the ion steady state in PAC. It is superior to earlier BCL-2 inhibitors. Therefore, the side effects of ABT-199 when used in the treatment of leukemia are relatively low. In recent years, it has been clinically discovered that a deacetylase inhibitor chidamide (CS055) combined with ABT-199

treatment can ensure cell viability and enhance ABT-199 activity (Chen, et al. 2020, Lucantoni, et al. 2018). In breast cancer treatment, selective inhibitors of BCL-2 and BCL-Xl slow down the synthesis of ATP and lethal cancer cells (Szlavik, et al. 2020). However, the overexpression of the drug in certain lymphoid malignancies is caused by drug resistance. Through the whole genome screening of human acute myeloid leukemia (AML), it is known that the mitochondrial structure causes a sensitive response to ABT-199. In the end, Kristina et al. proved that mitochondrial chaperone protein (CLPB) directly interacts with the main regulator of mitochondrial dynamics (OPA1) (Chen, et al. 2019). It is possible that evasion of ABT-199 resistance can be achieved by targeting mitochondria.

3 CONCLUSIONS

BH-3 protein plays an indispensable role in cell apoptosis by selectively binding to BCL-2 family anti-apoptotic proteins and inducing apoptosis. ABT-737, ABT-263, and ABT-199 promote the oligoylation of BAX and BAK and, eventually, the apoptosis of cancer cells. They have shown efficacy in some cancer cases and play an indispensable role in regulating apoptosis therapy. S63845, S64315, and VU661013 are all bH-3 mimics that inhibit BCL-1 and reduce the amplification of AML cell lines. A-1331852 is a practical, oral selective inhibitor of BCL-XL. ApoG2 study provides new ideas for nasopharyngeal carcinoma, respectively. Each of these drugs has its advantages but is not sufficient on its own because of the complexity of cancer and apoptotic procedures. Clinical studies have shown that combinations of drugs that impede BCL-2 and McL-1 proteins can improve treatment outcomes, such as the combination of S63845 and ABT-199, which can prolong the life of patients. This suggests that BH-3 mimics still have a promising application in cancer treatment.

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