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Scientific Publishing

The Role of the Pro-Apoptotic Protein Bax in Colon Cancer

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Abstract: Bax is a pro-apoptotic member of the Bcl-2 family that plays a crucial role in the regulation of apoptosis. Bax can be activated by various stimuli and translocate to the mitochondria, where it induces cytochrome c release and caspase activation. Bax expression or activity can be altered in colon cancer cells, resulting in resistance to apoptosis and tumor progression. This article summarized the molecular mechanisms of Bax activation and function in apoptosis, the alterations of Bax expression or activity in colon cancer cells, and the potential therapeutic implications of targeting Bax or its regulators in colon cancer.

Keywords: Bax; Apoptosis; Colon Cancer

Introduction

Apoptosis is a programmed cell death process that eliminates unwanted or damaged cells in a controlled manner (Li et al., 2021). Apoptosis is essential for maintaining tissue homeostasis and preventing tumorigenesis. Dysregulation of apoptosis can lead to various diseases, such as cancer.

The Bcl-2 family is a group of proteins that regulate apoptosis by controlling the permeability of the mitochondrial outer membrane. The Bcl-2 family consists of anti-apoptotic proteins (such as BCL-2), pro-apoptotic BH3-only proteins (such as BID and BIM) and pro-apoptotic effector proteins (such as BAX and BAK) members that share homology in one or more of the four Bcl-2 homology (BH) domains. The interaction between BCL-2 family members determines the life and death of cells.

Bax is one of the most studied pro-apoptotic members of the Bcl-2 family. It plays an important role in the suppression of tumorigenesis by inducing apoptosis in response to various stresses (Spitz & Gavathiotis, 2022). However, cancer cells often develop mechanisms to evade apoptosis by altering Bax expression or activity.

Colon cancer is one of the most common types of cancer worldwide and has a high mortality rate. Colon cancer can be classified into two major subtypes based on the microsatellite status: microsatellite stable (MSS) and microsatellite unstable (MSI). Microsatellites are short tandem repeats of DNA sequences that are prone to replication errors. MSI tumors have a high frequency of mutations in microsatellite regions due to defects in DNA mismatch repair genes. MSI tumors account for about 15% of sporadic colon cancers and 90% of hereditary nonpolyposis colon cancers (HNPCC). MSI tumors have distinct molecular and clinical features compared to MSS tumors, such as better prognosis, resistance to 5-fluorouracil-based chemotherapy, and increased immune infiltration.

1. The Molecular Mechanisms of Bax Activation and Function in Apoptosis

Apoptosis is divided into the extrinsic (also called death receptor) pathway and the intrinsic (also called mitochondrial) pathway. The pro-apoptotic protein Bax is mainly involved in the mitochondrial apoptosis pathway (Figure 1). The mitochondrial apoptosis pathway is an endogenous apoptosis mechanism that mainly involves the following steps (Ichim & Tait, 2016):

- BCL-2 homology domain 3 (BH3)-only protein is activated after cells are exposed to internal or external apoptotic stimuli (such as DNA damage, oxidative stress, growth factor loss).
- BH3-only protein activates pro-apoptotic members of the Bcl-2 family of proteins, such as Bax and Bak, and interacts with anti-apoptotic members, such as Bcl-2, leading to mitochondrial outer membrane permeabilization (MOMP).
- The permeability of the outer mitochondrial membrane increases, resulting in a decrease in mitochondrial membrane potential and the release of a series of apoptotic factors, such as cytochrome c and SMAC.
- Cytochrome c binds to Apaf-1, forms apoptotic bodies in the presence of ATP, recruits and activates procaspase-9, and forms

caspase-9 holoenzyme.

- The caspase-9 holoenzyme further activates effector caspases, such as caspase-3 and caspase-7, initiating the caspase cascade reaction, cleaving a variety of substrates in the cell, leading to the destruction of the cell nucleus and cytoskeleton, forming typical apoptotic morphology feature.
- SMAC is released from mitochondria, binds to IAPs, and inhibits the inhibitory effect of IAPs on caspase, thus indirectly promoting apoptosis.

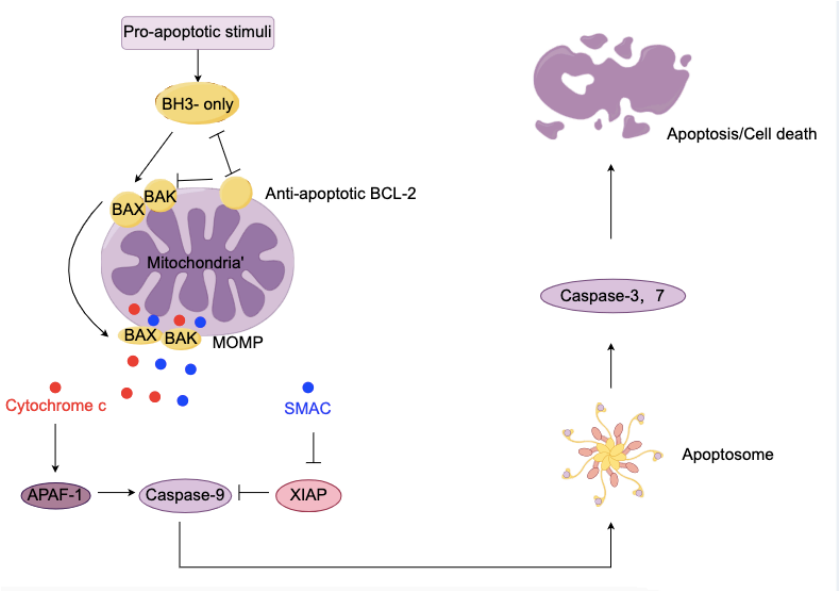

Figure 1. Mitochondria apoptotic signalling pathways

2. Alterations of Bax Expression or Activity in Colon Cancer Cells for Tumor Development and Progression

Bax expression or activity can be altered in colon cancer cells, resulting in resistance to apoptosis and tumor progression. The alterations of Bax expression or activity can be classified into two categories: genetic alterations and epigenetic alterations (Liu et al., 2016).

Genetic alterations refer to mutations or deletions that affect the structure or function of Bax. The most common genetic alteration of Bax in colon cancer is the deletion of one guanine in the microsatellite region of exon 3, which results in a frameshift and a premature stop codon. This mutation leads to the loss of Bax protein expression and function, which confers resistance to apoptosis and contributes to tumor progression. This mutation occurs predominantly in MSI colon cancers due to defects in DNA mismatch repair genes. The frequency of this mutation ranges from 30% to 70% in MSI colon cancers, depending on the study population and the detection method. However, alternative splicing can rescue the expression of a full-length Bax protein by skipping exon 2, which restores the reading frame. This Bax isoform is called BaxΔ2 and was first discovered in colon cancer patients with MSI. Unlike the parental Baxα, BaxΔ2 induces apoptosis through a non-mitochondrial pathway that involves caspase-8 activation. BaxΔ2 expression has been detected in various human tissues, especially in the colon, where it shows an inverse correlation with tissue malignancy. The mechanism of alternative splicing of Bax is not fully understood, but it may be influenced by factors such as splicing factors, RNA-binding proteins, or microRNAs.

Epigenetic alterations refer to modifications that affect the expression or activity of Bax without changing its sequence. The most common epigenetic alteration of Bax in colon cancer is the methylation of its promoter region, which results in the silencing of its transcription. Methylation of Bax promoter has been observed in both MSS and MSI colon cancers, but more frequently in MSS colon cancers. The frequency of methylation of Bax promoter ranges from 10% to 60% in colon cancers, depending on the study population and the detection method. Methylation of Bax promoter can be influenced by factors such as DNA methyltransferases, histone deacetylases, or microRNAs. Methylation of Bax promoter can be reversed by treatment with demethylating agents, such as 5-aza-2'-deoxycytidine (5-AZA) or zebular-

ine, which can restore Bax expression and induce apoptosis in colon cancer cells.

Other epigenetic alterations of Bax in colon cancer include post-translational modifications or interactions with other proteins that modulate its stability, localization, conformation, or interaction with other proteins.

3. Potential Therapeutic Implications of Targeting Bax or Its Regulators in Colon Cancer

Bax is a potential therapeutic target for colon cancer, as restoring or enhancing its expression or activity can induce apoptosis and inhibit tumor growth. Several strategies have been developed to target Bax or its regulators in colon cancer, such as gene therapy, small molecules, peptides, antibodies, or nanomaterials (Walensky, 2019).

Gene therapy involves the delivery of exogenous genes that encode Bax or its activators to colon cancer cells using viral or non-viral vectors. For example, adenovirus-mediated transfer of Bax gene can induce apoptosis and suppress tumor growth in colon cancer xenograft models. Adenovirus-mediated transfer of Bid gene can also induce apoptosis and suppress tumor growth in colon cancer xenograft models. However, gene therapy faces several challenges, such as low efficiency, immunogenicity, toxicity, or specificity.

Small molecules are organic compounds that can modulate the expression or activity of Bax or its regulators by binding to their targets with high affinity and specificity. For example, ABT-737 is a small molecule that mimics the BH3 domain of Bad and binds to anti-apoptotic proteins, such as Bcl-2, Bcl-xL, and Bcl-w, displacing Bax from them and activating it. ABT-737 can induce apoptosis and inhibit tumor growth in colon cancer cell lines and xenograft models. However, ABT-737 has limited efficacy against Mcl-1-expressing colon cancer cells, which can be overcome by combining it with other agents, such as obatoclax, sorafenib, or navitoclax. TW-37 is another small molecule that mimics the BH3 domain of Bad and binds to anti-apoptotic proteins, such as Bcl-2, Bcl-xL, and Mcl-1, displacing Bax from them and activating it. TW-37 can induce apoptosis and inhibit tumor growth in colon cancer cell lines and xenograft models. However, TW-37 has low solubility and bioavailability, which can be improved by using nanocarriers, such as liposomes, micelles, or nanoparticles.

Peptides are short sequences of amino acids that can modulate the expression or activity of Bax or its regulators by binding to their targets with high affinity and specificity. For example, BH3 peptides are synthetic peptides that mimic the BH3 domain of BH3-only proteins and bind to anti-apoptotic proteins, displacing Bax from them and activating it. BH3 peptides can induce apoptosis and inhibit tumor growth in colon cancer cell lines and xenograft models. However, BH3 peptides have low stability, permeability, and specificity, which can be enhanced by using modifications, such as D-amino acids, cyclization, stapling, or conjugation.

Antibodies are proteins that recognize and bind to specific antigens on the surface or inside of cells. Antibodies can be used to activate Bax or inhibit its inhibitors in colon cancer cells. For example, 6A7 is an antibody that recognizes and binds to the inactive form of Bax and induces its conformational change and activation. 6A7 has been shown to induce apoptosis and sensitize colon cancer cells to 5-fluorouracil or cisplatin. Alternatively, antibodies can be used to inhibit the expression or activity of epigenetic modifiers that repress Bax expression in colon cancer cells. For instance, EZH2 is an antibody that recognizes and binds to EZH2, which is a subunit of PRC2 that catalyzes H3K27 methylation of the Bax promoter, resulting in its silencing and apoptosis resistance. EZH2 has been shown to induce apoptosis and enhance the efficacy of 5-fluorouracil or irinotecan in colon cancer cells.

Nanoparticles are particles that have a size range of 1-100 nanometers and can carry various cargoes, such as genes, drugs, peptides, or antibodies. Nanoparticles can be used to deliver Bax or its activators or inhibitors into colon cancer cells with high specificity and efficiency. For example, liposomes are nanoparticles that consist of lipid bilayers that can encapsulate hydrophilic or hydrophobic substances. Liposomes can be used to deliver Bax gene, ABT-737, SAHB, or 6A7 into colon cancer cells and induce apoptosis and chemosensitization.

4. Conclusion & Discussion

Bax has shown amazing potential in targeting Bax or its regulators in colon cancer. However, there are still many unanswered questions and challenges that need to be addressed in future studies. For example, what are the molecular determinants and dynamics of Bax oligomerization and pore formation? How do different Bax isoforms interact with each other and with other proteins? How do different microenvironments and signaling pathways affect Bax expression or activity in colon cancer cells? How can we overcome the heterogeneity and

resistance of colon cancer cells to Bax-targeted therapies? How can we optimize the delivery and specificity of Bax-targeted therapies? How can we combine Bax-targeted therapies with other conventional or novel therapies for synergistic effects? These questions warrant further investigation and exploration to elucidate the role of Bax in colon cancer and to develop more effective and personalized treatments for colon cancer patients.

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