Japanese Journal of Gastroenterology and Hepatology

Review Article

Bcl-2 and SIRT3 Affect Tumor Cell Viability by Integrating Metabolism and Apoptosis

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Received: 29 July 2020

Accepted: 08 Aug 2020

Published: 10 Aug 2020

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1. Abstract

While apoptotic tolerance caused by B cell lymphoma 2 (Bcl-2) over expression is one of the important underlying mechanisms of the decreased apoptotic sensitivity of tumor cells, only targeting Bcl-2 as an anticancer therapy has shown limited clinical benefit, which may be related to the non-apoptotic protective effects of Bcl-2, such as its roles in regulating endoplasmic reticulum stress and autophagy. Recently, it was suggested that Bcl-2 plays dual roles in regulating cellular metabolism and apoptosis. However, Bcl-2 inhibitors only alter metabolism by down regulating the expression glycolytic enzymes, while glycolysis inhibitors increase the sensitivity of cells to apoptosis by Bcl-2 inhibitors. Bcl-2 is primarily distributed in mitochondria, which have the dual functions of being intracellular energy factories and executers of death signals. Surtuin 3 (SIRT3) is an important mitochondrial deacetylase that regulates many mitochondrial metabolic pathways. SIRT3 enzymatic activity is dependent on the NAD+/NADH ratio. Inhibiting Bcl-2 alters tumor cell metabolism and decreases the NAD+/NADH ratio, which activates SIRT3. SIRT3 then modifies glycolysis-related proteins by deacetylation, which affects ROS levels, HIF-1α activity, and the localization of Hexokinase II, which is the key glycolytic enzyme. Together, these effects reverse (inhibit) the metabolic reprogramming of tumor cells and enhance their apoptotic sensitivity. Herein, we discuss this new model that suggests that integration of Bcl-2 and SIRT3 signaling regulates apoptosis through glycolysis and mitochondrial oxidative phosphorylation.

2. Keywords: Bcl-2; SIRT3; Metabolic reprogramming

3. Introduction

Apoptosis escape and metabolic reprogramming are two hallmarks of malignant tumors and are important targets for antitumor therapies. During cancer progression, proteins of the anti-apoptotic Bcl-2 family, which are primarily localized to mitochondria, are often highly expressed, and thus they are considered key genes for tumor cell signaling. Inhibiting the anti-apoptotic Bcl-2 proteins may enhance the sensitivity of tumor cells to chemotherapeutic drugs by increasing activation of the mitochondrial apoptotic pathway. Therefore, several BH3-only protein mimics (BH3 mimetics), such as HA14-1, GX15-070, BI-33, ABT-737, and S1, have been synthesized to target Bcl-2.These mimics compete with the endogenous anti-apoptotic proteins Bcl-2, Bcl-XL, Bcl-w and Mcl-l, ultimately inducing apoptosis through pro-apoptotic proteins, such as Bax and Bak, and they have been shown to exert antitumor effects. However, human clinical trials have shown limited clinical efficacy [1], indicating that only inhibiting Bcl-2 does not achieve the therapeutic goals.

Bcl-2 is not only involved in apoptosis, but also in a variety of cellular physiological processes, including metabolism. The reprogramming of cellular metabolism by oncogenes may also affect cell survival/death.SIRT3 is the main mitochondrial deacetylase and targets several enzymes in-

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volved in important metabolic processes. As a key oncogene, studies have shown that when Bcl-2 is highly expressed, the nicotinamide adenine dinucleotide (NAD+/NADH) ratio decreases; high glycolytic activity can also reduce the NAD+/NADH ratio; thus, both inhibit SIRT3 activity. The decrease in SIRT3 reduces the transformation of NADH to NAD+, further reducing SIRT3 activity, suggesting Bcl-2 regulates SIRT3 through glycolysis. Because SIRT3 has been proved to be an important regulator of the balance between glycolysis, anabolism, and mitochondrial oxidative metabolism, we speculate that SIRT3 may be a key regulator of glucose metabolism and apoptosis.

This review focuses on the integration of Bcl-2 and SIRT3 in tumor cell metabolism and apoptosis, with the goals of uncovering the mechanisms of apoptotic tolerance and improving the sensitivity of cancer cells to apoptosis.

3.1. Roles of the Bcl-2 Protein Family in Cancer Cell Apoptosis, Autophagy/Endoplasmic Reticulum Stress, and Metabolic Reprogramming

3.1.1. The Bcl-2 Protein Family and Cancer Cell Apoptosis: Apoptotic escape is one of the signs of cancer, and therefore most anticancer treatments aim to induce programmed cell death by activating related signaling networks [2]. The Bcl-2 protein family is the most important regulatory node of the mitochondrial apoptosis pathway. Cancer cell apoptosis can be promoted by decreasing Mcl-1 and Bcl-xl expression [3, 4]. These proteins block apoptosis by binding and isolating Bax/Bak proteins to release cytochrome C into the cytoplasm, and by binding and chelating the BH3-only pro-apoptotic members (Bim, Noxa, Puma, Bad, Bik, and Bid) to dissociate Bak and Bax [5]. Therefore, the balance between anti-apoptotic and pro-apoptotic proteins determines cell death or survival, and the synergistic action of Bax and Bak is at the core of the mitochondrial apoptosis pathway (Figure 1) [6, 7].

Bcl-2 not only participates in the mitochondrial apoptosis pathway, but is also localized to the endoplasmic reticulum where it regulates apoptosis mediated by endoplasmic reticulum stress. While the BH3-only protein mimics (BH3 mimetics) synthesized to block the anti-apoptotic protein Bcl-2 are needed in high concentration to exert their activity on cancer cells, many of them are cytotoxic, which limits clinical applications. Therefore, one option to improve the efficacy of Bcl-2 inhibitors is to use them in combination with other inhibitors, including metabolic inhibitors [1].

3.1.2. The Bcl-2 Protein Family and Endoplasmic Reticulum Stress/Autophagy: While chemotherapeutic drugs can induce apoptosis in tumor cells, these effects are accompanied by increased autophagy. Autophagy can reduce the level of endoplasmic reticulum stress by clearing misfolded proteins.

Many Bcl-2 family members, including Bcl-2, Bax, Bak, and Bik/Nbk, are localized to the endoplasmic reticulum where they control autophagy, another pathway that regulates cell survival. Autophagy can reduce cellular stress by removing sensors of the endoplasmic reticulum Unfolded Protein Response (UPR) or by eliminating abnormal proteins in the endoplasmic reticulum [8]. Inhibiting this relief of endoplasmic reticulum stress from autophagy may be a way to enhance the efficacy of antineoplastic drugs.

Studies have demonstrated that the BH3 mimetic S1 induces Bax/Bak-dependent apoptosis in many tumor cell lines through the endoplasmic reticulum stress pathway and autophagy [9]. Specifically, in U251 cells, S1 blocks the connection between endoplasmic reticulum stress signaling and the destruction of Bcl-2/Beclin1. Additionally, the autophagy inhibitors 3-methyladenine (3-MA) and chloroquine (CQ) increase the levels of apoptosis induced by S1. This increase suggests that autophagy plays an important role in S1-mediated cell death, at least in U251 cells.

ABT737 is another BH3 mimetic and significantly increases the sensitivity of cancer cells to cisplatin, which is related to its effects on the expression and subcellular localization of Bcl-2.Additionally, ABT737 promotes p62 aggregation in cells, which is associated with mitochondrial fission; inhibiting p62 alters Drp1 protein levels and mitophagy [10]. These results demonstrate that the Bcl-2 inhibitors S1, ABT737, and likely other BH3 mimetics, regulate the endoplasmic reticulum stress/autophagy pathway in addition to the mitochondrial apoptosis pathway [11, 12].

Thus, this class of drugs enhances the cytotoxicity of oxidative stress and causes autophagy/endoplasmic reticulum imbalance, while also significantly enhancing sensitivity to antineoplastic drugs by affecting mitochondrial dynamics (including promoting mitochondrial fission and excessive mitophagy). Further elucidating the mechanisms of autophagy induced by endoplasmic reticulum stress that are regulated by Bcl-2 at the molecular level will be important for the development of future antineoplastic drugs.

3.1.3. Role of the Bcl-2 Protein Family in Reprogramming Glucose Metabolism in Cancer Cells: The German scientist Otto Warburg found that tumor cells convert glucose into lactic acid through glycolysis even under sufficient oxygen conditions, in a phenomenon now called the Warburg effect [13]. In the past, it was believed that this reprogramming of glucose metabolism in tumor cells was regulated by complex factors such as the tumor microenvironment and altered gene expression. These factors were thought to increase the expression and activity of glycolytic enzymes and glucose transporters. It was also hypothesized that mitochondrial aerobic oxidation was decreased through intrinsic mechanisms, such as decreased utilization of pyruvate, disturbance of the tricarboxylic acid cycle, or respiratory damage to the electron transport chain. Other studies showed that pyruvate dehydrogenase (PDH) kinase 1 (PDK1) inhibitors could restore the activity of PDH and mitochondrial oxidative phosphorylation. When tricarboxylic acid cycle activity is completely or partially lost, it leads to increased glycolysis, which decreases

the sensitivity of cells to apoptosis [13]. During tumorigenesis and the development of metastases, changes in intracellular signaling pathways and the extracellular microenvironment make metabolic reprogramming and the ability to quickly balance glycolysis and mitochondrial metabolism a key to cancer cell survival. Metabolic reprogramming by cancer cells may prevent tumor cells from death; thus, reversing (inhibiting) metabolic reprogramming of cancer cells could improve their apoptotic sensitivity.

Bcl-2 and Bcl-xL regulate mitochondrial function through adenine nucleotide translocator (ANT), voltage-dependent anion channels (VDACs), and hexokinase/glucokinase (HK), which plays an intermediary role in metabolism and affects cell survival [14, 15]. Bax, Bad, and Bcl-2 also regulate Ca2+ homeostasis in the endoplasmic reticulum. Ca2+ activates p53, which is not only a powerful pro-apoptotic factor, but also and inhibitor or aerobic glycolysis in tumor cells, which plays a homeostatic role in metabolism [16]. Moreover, the Bcl-2 inhibitors S1 and ABT737 decrease the transcription of glycolysis-related genes and increase the levels of gluconeogenesis- and tricarboxylic acid cycle-related genes in ovarian cancer SKOV3 cells, while the glycolysis inhibitor 2-DG increased the levels of apoptosis induced by the Bcl-2 inhibitors S1 and ABT737 [17, 18].

4. SIRT3 Participates in Tumor Cell Energy Metabolism

SIRT3 is a member of the family of lysine deacetylases and/or single ADP ribosyl-transferases (Sirtuins). SIRT3 activity depends on NAD+, which is primarily in the mitochondria [19-21]. Compared with class I and II histone deacetylases, the nutritional sensitivity of the deacetylation activity of Sirtuins plays a special role in maintaining metabolic homeostasis [22]. NAD+ is the main electron receptor for a variety of metabolic pathways; thus, cellular metabolism is extremely sensitive to intracellular NAD+ levels [23]. Changes in the NAD+/NADH ratio can alter SIRT3 activity [24].Aerobic glycolysis by tumor cells can produce nicotinamide adenine dinucleotide phosphate (NADPH) through the pentose phosphate pathway (PPP). Increased NADPH reduces SIRT3 activity while maintaining cellular antioxidant activity (Figure 1).

SIRT3 also regulates glycolysis. In SIRT3-deleted mouse embryonic fibroblasts, the deacetylation of mitochondrial manganese superoxide dismutase (SOD) decreased, which decreased ROS scavenging. Increased ROS can inhibit prolyl hydroxylase activity and reduce the degradation of hypoxia inducible factor (HIF)-1α, resulting in increased HIF-1α activity [25]. HIF-1α activates the transcription of a series of glycolysis-related enzymes and promotes glycolysis. Moreover, SIRT3 expression is decreased in breast cancer, and its loss is associated with the upregulation of HIF-1α target genes.Additionally, SIRT3 over expression inhibits the proliferation of breast cancer cells and glycolysis [26-28]. This effect was also found in human colon cancer and osteosarcoma cells (Figure 1) [29].

Hexokinase II (HK-II) is a key enzyme in glycolysis, and it also functions in transcriptional regulation and apoptosis induction [30]. SIRT3 knockout cells showed increased expression of HK-II.Studies have also shown that SIRT3-mediated deacetylationof cyclophile D leads to the dissociation of HK-II from mitochondria and the VDAC complex [31]. Loss of HK from this complex activates apoptosis and inhibits glycolysis in many cancer cells. It has been suggested that SIRT3 may regulate apoptosis and metabolism by affecting HK-II localization (Figure 1).

Contrary to nuclear and cytoplasmic proteins, acetylation activates

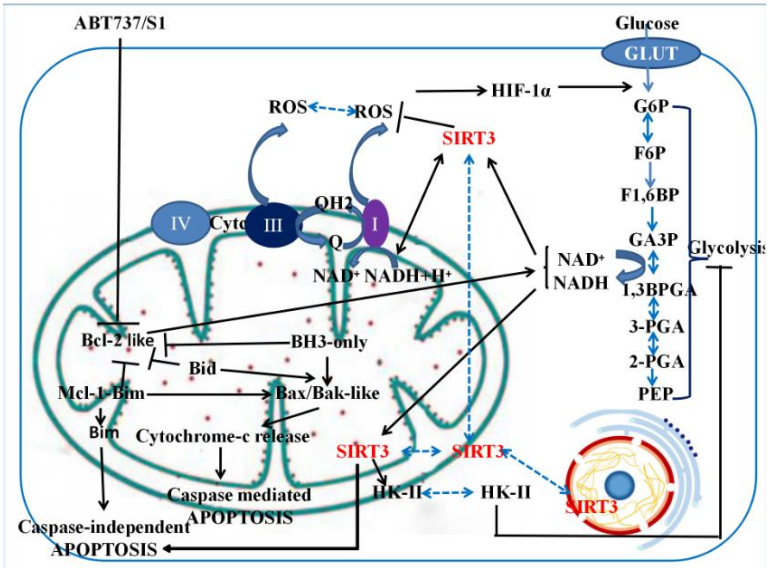

Figure 1: The NAD+/NADH ratio is high when Bcl-2 expression is decreased, while high glycolytic activity decreases the NAD+/NADH ratio, which inhibits SIRT3 activity. This decreased SIRT3 activity reduces the transformation of NADH to NAD+, which further reduces SIRT3 activity. At the same time, SIRT3 promotes apoptosis, and decreased SIRT3 activity leads to increased oxidation, HIF-1α activity, and the transcription of HIF-1α-activated genes, which include many glycolytic enzymes; thus, glycolysis is promoted. SIRT3 also causes the dissociation of Hexokinase II from mitochondria, which activates apoptosis and inhibits glycolysis.

mitochondrial proteins [32]. As the main deacetylation enzyme for mitochondrial proteins, SIRT3 can promote the deacetylationof mitochondrial respiratory chain complex, increase mitochondrial oxidative phosphorylation, and increase ATP production.

5. Bcl-2 and SIRT3 Participate in Metabolic Reprogramming to Determine Cell Fate

It has been found that the NAD+/NADH ratio is decreased by 33%–50% in Bcl-2 over expressing cells compared with the original levels [33]. High glycolysis activity can reduce the NAD+/NADH ratio, and thus inhibit the activity of Sirtuins [34]. The decreased activity of Sirtuins can reduce the deacetylation of mitochondrial protein, inhibit effective electron transport by the electron transport chain, and reduce the transformation of NADH to NAD+, which further reduces the activity of Sirtuins [26]. Additionally, it has been found that SIRT3 plays a pro-apoptotic role in human epithelial carcinoma HCT116 cells in which Bcl-2 has been silenced [35].

S1 induced apoptosis and inhibited mitochondrial respiration in human ovarian cancer cells. S1 also effectively increased the intracellu-

lar NAD+/NADH ratio and decreased the Oxygen Consumption Rate (OCR) of ovarian cancer cells, which may contribute to SIRT3 activation. 2-DG is an inhibitor of glycolysis. Combining 2-DG and S1 further promoted apoptosis by enhancing SIRT3 expression [17].

The previous experiments in our laboratory on the apoptosis tolerance of drug-resistant ovarian cancer cells showed high expression of Bcl-2, but low expression of SIRT3. We also found that Bcl-2 promoted glycolysis in ovarian cancer cells, indicating that low SIRT3 expression may be one of the reasons for apoptosis resistance in ovarian cancer cells. Therefore, SIRT3 may be a tumor suppressor gene for drug-resistant ovarian cancer cells.

Together, these data suggest that there is a close relationship between Bcl-2 and SIRT3 during the process of chemotherapeutic drug-induced apoptosis (Figure 1). Therefore, we believe that inhibiting the Bcl-2-induced up-regulation of SIRT3 and restoring mitochondrial metabolic balance could reverse (inhibit) metabolic reprogramming in tumor cells.

6. Discussion

Traditional antitumor metabolic drugs have the characteristics of low specificity, many adverse reactions, and drug resistance. In a study of metformin combined with SIRT3 for antitumor treatment, the doses used were too high for clinical application; thus, it is necessary to find therapeutic strategies that reduce metformin concentrations [36].

It was recently shown that Bcl-2 has dual roles in regulating cellular metabolism and apoptosis. Bcl-2 inhibitors must bind to the target Bcl-2 family proteins at an appropriate concentration for clinical application. Moreover, there is an irreconcilable contradiction between high affinity and broad-spectrum targeting, which may also increase toxicity.

SIRT3 regulates a variety of metabolic pathways, and thus this review focused on integrating the metabolic and apoptotic roles of Bcl-2 and SIRT3. Bcl-2 inhibits SIRT3 activation, which affects oxidation levels, HIF-1α, and HK-II localization. We speculate that inhibiting tumor cell metabolic reprogramming could enhance sensitivity to apoptosis. Finally, this review summarized our understanding of the complex biological functions of the Bcl-2 family proteins and SIRT3 in tumor cells and provided theoretical support for experimental research to improve the sensitivity of tumor cells to chemotherapy.

7. Acknowledgments

This project was supported by the National Natural Science Foundation of China (Grant number: 81772794, and 81672948), Jilin Provincial Research Foundation for Health Technology Innovation (2019J009), Jilin Provincial Research Foundation for the Development of Science and Technology Projects (20191004004TC), Jilin provincial Industrial Innovation Project (2018C052-7), and also supported by 'the Fundamental Research Funds for the Central Universities, JLU'.

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