5 - 60 Aberrant Bcl-x Splicing in Cancer: From Molecular Mechanism to Therapeutic

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Apoptosis regulator Bcl-extra (Bcl-x), also named BCL2L or BCL2L1, is a typical example of apoptotic response gene impacted by splicing. It is an essential member of B-cell lymphoma 2 (Bcl-2) apoptosis family that regulates cell fate[1,2]. Bcl-x nascent transcripts are alternatively spliced and mainly encode two antagonistic isoforms. The long isoform Bcl-xL blocks apoptosis by inhibiting pro-apoptotic counterparts of Bcl-2 family, whereas the short isoform Bcl-xS can promote apoptosis[2]. An increasing body of data suggests that dysregulated expression of Bcl-x apoptotic isoforms contributes to multiple hallmarks of human cancers. For example, Bcl-xL level was strongly enhanced in cancer cells at the invasive forefront of human breast carcinomas and simultaneously acquired resistance to apoptotic stimuli[3,4]. However, Bcl-xS conferred the therapeutic sensitivity by decreasing the apoptosis threshold[5]. The ratio of pro-apoptotic Bcl-xS and anti-apoptotic Bcl-xL proteins plays a vital role in regulating the switch between cell life and death.

Chemotherapy

Plenty of evidence suggested that Bcl-xL-dependent apoptotic inhibition was the main reason that promoted chemotherapy resistance of tumors *in vitro* and *in vivo*[6,7]. A study on breast cancer showed that cells passed through EMT obtained therapeutic resistance by upregulating Bcl-xL transcripts. However, apparent apoptotic resistance was removed after deleting Bcl-xL[4]. Bcl-xL was also found to mediate doxorubicin resistance of breast cancer through the Ring finger protein 6/Estrogen receptor α/Bcl-xL pathway[8]. Inhibiting Bcl-xL expression in breast cancer cells enhanced the cytotoxicity and apoptosis induced by T-DM1[9]. Additionally, increased CXCR4 expression in ovarian cancer induced cisplatin resistance through promoting Bcl-xL/S[6]. Upregulated Bcl-xL expression was also found to be involved in resistance to therapy targeting Bcl-2 in mantle-cell lymphoma and Acute Myelocytic Leukemia[10]. Regarding melanoma, it has been demonstrated that forced expression of ectopic Bcl-xL converted drug-sensitive cell lines into drugresistant ones[11]. However, vivo-Morpholino (vMO) antisense oligomers that used to upregulate Bcl-xS expression but decrease Bcl-xL in chronic myeloid leukemia (CML) increased growth inhibition and apoptotic sensitivity of imatinib mesylate-resistant CML cells[5]. Similarly, overexpressed Bcl-xS in human breast carcinoma cells induced a remarkable increase in sensitivity chemotherapy agents, but did not affect cell viability by itself[12].

Radiotherapy

The splicing favor of Bcl-xL contributed to long-term radiotherapy resistance. Clinical data showed that Bcl-xL was expressed by about 91% of laryngeal cancer patients resistant to radiotherapy, suggesting a critical function of Bcl-xL in radiotherapy[13]. Streffer[14,15] *et al.*, found that glioma cell lines with high Bcl-xL expression had higher ED50 (2.9 ± 0.8 Gy) than cell lines with lower Bcl-xL. However, no association with radiosensitivity was observed for the expression levels of Bcl-xS. Highly expressed Bcl-xL was also found to cause radiation resistance of osteosarcoma cells with both low and high metastasis level, and Bcl-xL downregulation could significantly enhance radiation cytotoxicity of osteosarcoma cells[16]. Moreover, inhibiting the expression level of Bcl-xL were suggested to reverse radioresistance and regulate radiation-induced apoptosis of mesothelioma, breast cancer, prostate cancer, colorectal cancer as well as non-small cell lung cancer[17−20]. In addition to therapeutic effects, irradiation was well known to induce increased invasiveness and metastasis of cancer cells. Ho, *et al.*, demonstrated that the expression of Bcl-x was elevated after irradiation, which promoted the malignant actions of lung cancer cells[21]. A recent study also suggested that upregulated Bcl-xL induced invasion of cancer cells that underwent sublethal doses of irradiation by stimulating respiratory complex I and increasing additional ROS production, which might be involved in the local recurrence or distal metastasis of somne patients after radiotherapy[22]. Interestingly, the expression of Bcl-xL could enhance energy metabolism and prevent oxidative stress, which might be involved in the alleviation of mitochondrial oxidative stress induced by radiation[23]. These results provided a wealth of evidence that inhibition the endogenous expression of Bcl-xL might promote both radiation sensitization and radiation protection.

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5 - 61 SPP1 as a Potential Biomarker for Immune Microenvironment of Glioblastoma

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Glioblastoma (GBM) is the most common and aggressive primary brain tumor, accounting for about 14.5% of malignant brain and other Central Nervous System (CNS) tumors[1]. Among primary malignant brain and other CNS tumors, only GBM had the lowest median survival about 8 months[2]. The GBM tumor microenvironment (TME) is unique due to its special cellular components, which contributes to the brain being widely recognized as a relatively immunologically privileged organ. Immune-privileged organs tightly regulate immune responses, which results in a natural immunosuppressive environment. Macrophages constitute the main body of infiltrating immune cells and are thought to have preneoplastic lesions and immunosuppressive effects[3]. It is very important to explore the role of macrophages related pathways in the occurrence and development of GBM.

Here, we firstly developed an immune-related prognostic model and verified its ability to significantly distinguish between good and bad outcomes of patients, finding that SPP1 has good diagnostic and prognostic ability in GBM (Fig. 1(a)∼(b)). Subsequently, we analyzed the correlation of GBM immune microenvironment with the prognostic model. It was found that there was a significant correlation between the model and the immune microenvironment, especially model gene SPP1 and macrophages (Fig. 1(c)). Finally, we explored the difference of SPP1 pathway between adjacent tissues and tumor tissues, and found that SPP1 pathway played an important role in tumor tissues (Fig. 1(b)). Subsequently, we visualized the interacting receptor and ligand pairs in the SPP1 pathway in peritumoral and tumor tissues, and found that the main receptor and ligand pairs that played a role in tumor tissues were SPP1-CD44 and SPP1-interferon, especially the SPP1-CD44 receptor and ligand pair contributed most to the SPP1 pathway (Fig. 1(e)).

In conclusion, SPP1 pathway plays an important role in the occurrence and development of GBM. Therefore, the efforts to explore mechanism of SPP1 pathway in glioblastoma are further needed.