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Review

BLID: A Novel Tumor-Suppressor Gene

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BLID (BH3-like motif containing, cell death inducer), also known as breast cancer cell 2 (BRCC2), was first reported in the human breast cancer cell line in 2004. BLID is a BH3-like motif containing apoptotic member of the Bcl-2 family. Recently, the BLID tumor-suppressor roles have been fully established. Several studies have found that BLID is frequently downregulated in many human cancers and the downregulation is often associated with tumor progression. Multivariate analysis indicated that BLID is an independent prognostic factor for overall survival and distant metastasis-free survival. Moreover, BLID can inhibit breast cancer cell growth and metastasis and promote apoptosis. BLID can regulate the expression of various tumor-related genes and proteins, such as AKT and MMP. In this review, we provide an overview of current knowledge concerning the role of BLID in tumor development and progression. To our knowledge, this is the first review about the role of this novel tumor-suppressor gene in tumor development and progression.

Key words: BH3-like motif containing, cell death inducer (BLID); Cancer; Tumor-suppressor gene (TSG)

INTRODUCTION

Cancer is still a major public health issue, with one in four deaths in the world attributable to the disease (1–3). Cancers encompass multiple entities of complex and multifactorial diseases, each having its own specific etiology and genomic, histological, and clinical characteristics. Despite recent advances in treatment strategies, effective clinical management remains elusive due to intratumoral heterogeneity and therapeutic resistance (4–6). Therefore, it is necessary to elucidate the molecular mechanisms involved in cancer in order to identify novel markers for effective diagnosis and treatment.

A gene is defined as a tumor-suppressor gene (TSG) if its mutation predisposes the animal to cancer (7). A TSG encodes for a protein that blocks tumor development, negatively regulating cell proliferation, migration, and invasion and/or contributing to the maintenance of genome stability (8). Tumor suppressors usually fall into two classes: gatekeepers and caretakers (9). Caretakers suppress cancer by repairing damaged DNA such as BRCA1/2 and MSH2, whereas gatekeepers suppress cancer by halting the cell cycle long enough to repair damaged DNA such as p53 and pRb (10–14).

Human BLID (BH3-like motif containing, cell death inducer), also known as breast cancer cell 2 (BRCC2), was first reported in the human breast cancer cell line in 2004 (15). BLID is an intronless gene that has been mapped to human chromosome 11q24.1 using fluorescence in situ hybridization (16). The longest predicted open reading frame (ORF) of BLID is 862 bp, and its mRNA encodes a protein that is 108 amino acids in length (17). Previous studies have shown that BLID was an independent prognostic factor for overall survival and distant metastasis-free survival in breast cancer. Furthermore, BLID inhibited breast cancer cell growth and metastasis in vitro and in vivo via downregulating AKT pathway (17). All these findings suggested that BLID might function as a new tumor suppressor. In this review, we will detail for the first time what is known about the BLID gene, its regulation of tumorigenesis, and its specific deregulations in human tumors.

STRUCTURAL FEATURES OF BLID

Cavalli et al. have precisely mapped the human chromosomal location of BLID to 11q24.1, using fluorescence in situ hybridization mapping (16). Recent findings from

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the genome-wide association studies (GWAS) have been pointing to novel disease-associated DNA regions, opening doors for new and more accurate targets for research and future therapies for many diseases (18). One of these new loci of these disease-associated DNA regions is the chromosome 11q24.1, which was highlighted as the strongly genetic susceptibility locus for many diseases, such as short stature, distinctive facial features, keratoconus, overweight, intellectual disability, osteogenesis imperfecta, and pathological myopia. Chromosome 11q24.1 was also the genetic susceptibility locus influencing lipid and lipoprotein levels (19–23). Remarkably, variants in this region have also been associated with several cancers: cervical cancer, chronic lymphocytic leukemia, monoclonal B-cell lymphocytosis, and breast cancer (21,24–26) (Fig. 1).

BLID was originally identified as a ~1.2-kb transcript in human breast carcinoma cells (15). The longest predictive ORF of BLID cDNA (327 bp) codes for a protein consisting of ~1 kDa predominately cytosolic protein (108 amino acids) (17).

STATUS OF BLID IN HUMAN CANCERS

Several studies have identified that BLID expression is decreased or lost in human tumors, which support a role for BLID as a tumor-suppressor gene.

A high frequency of loss of heterozygosity (LOH) of the BLID chromosomal region 11q24.1 has been found in early onset breast cancer and ovarian neoplasias, which also have a significant association with poor prognosis and reduced survival (27). This evidence suggests that this chromosomal region may harbor putative tumor-suppressor gene. Previous studies have shown that LOH at two microsatellite markers closely linked BLID gene on 11q24.1 in 50% of breast tumors from patients with early onset disease (age $\leq$ 40), compared to 21% and 32% of tumors from patients in aged 41–55 and $\geq$56-year-old groups, respectively. In addition, the odds of BLID loss for patients 40 years and younger were 3.7 times the odds of loss for patients aged 41–55 (95% CI, 1.1–13) (27). Broustas et al. indicated that BLID mRNA expression was significantly reduced in grade 3 relative to grades 1 and 2 breast cancer ($p=0.023$) (28). Cytoplasmic BLID immunoreactivity was absent in invasive ductal breast carcinomas (IDC). Lack of BLID expression was associated with younger age (median 40 years), African American ethnicity, tumor size, and triple-negative breast cancer (estrogen receptor negative, progesterone receptor negative, and human epidermal growth factor receptor 2 negative; all $p<0.005$). Significant correlations were observed between BLID loss and declines in overall, cause-specific, and local relapse-free survival (all $p<0.03$). Multivariate analysis indicated that BLID is an independent prognostic factor of distant metastasis-free survival (hazard ratio, 0.302; 95% confidence interval, 0.160–0.570, $p=0.0002$). It was also found that BLID can bind to Bcl-xL, and the binding was enhanced in cancer cells exposed to doxorubicin or cisplatin. Li et al. have found that BLID was strongly expressed in all normal breast tissues, and it became lower and weaker gradually in the progression from normal, UDH (usual ductal hyperplasia), ADH (atypical ductal hyperplasia), and DCIS (ductal carcinoma in situ) to breast cancer (29). Statistical analysis demonstrated a significant difference of BLID expression between proliferative and cancerous breast lesions. The data suggested that loss of BLID may contribute to the progression from intraductal proliferation lesions to breast cancer (Fig. 2).

TUMOR-SUPPRESSOR FUNCTIONS FOR BLID

Since its identification, BLID functions have been investigated in human cancer cell lines. Downregulation of BLID, as it occurs in human tumors, has given new insight in the understanding of BLID functions.

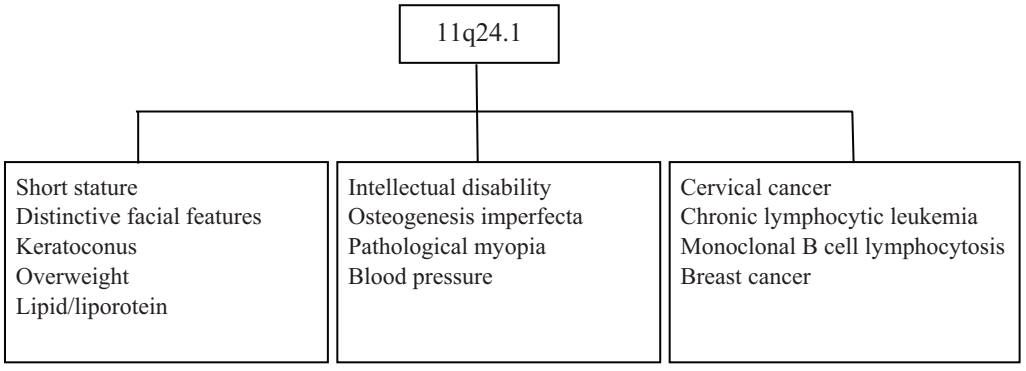

Figure 1. Chromosome 11q24.1 genomic region and the related disease. The chromosome 11q24.1 was highlighted as the genetic susceptibility locus for short stature, distinctive facial features, keratoconus, overweight, and intellectual disability, osteogenesis imperfecta, and pathological myopia. It also influences lipid and lipoprotein levels. Remarkably, variants in this region have also been associated with several cancers: cervical cancer, chronic lymphocytic leukemia, monoclonal B-cell lymphocytosis, and breast cancer.

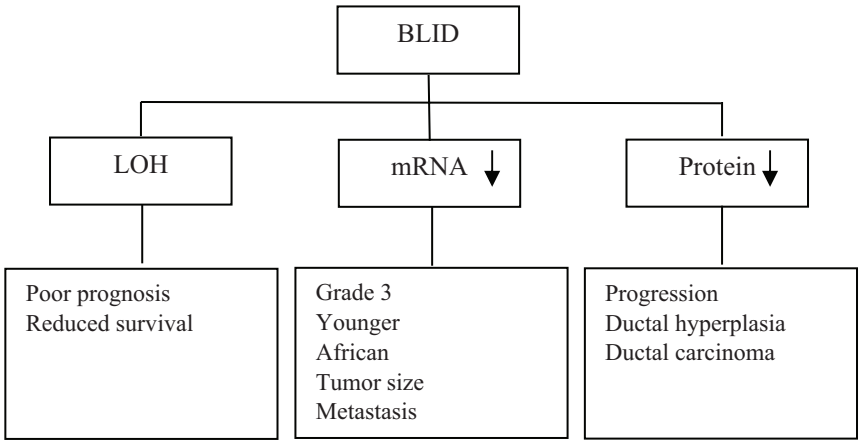

Figure 2. BLID status in human cancers. Loss of heterozygosity (LOH) of the BLID has been found in early onset breast cancer and ovarian neoplasias, associated with poor prognosis and reduced survival. BLID mRNA expression was significantly reduced in grade 3 compared to grades 1 and 2 breast cancer. Lack of BLID expression was associated with younger age, African American ethnicity, tumor size, and distant metastasis-free survival. BLID was strongly expressed in all normal breast tissues, but its expression became lower and weaker gradually in the progression from normal, UDH (usual ductal hyperplasia), ADH (atypical ductal hyperplasia), and DCIS (ductal carcinoma in situ) to breast cancer.

BLID could inhibit cell growth and metastasis in breast cancer cells in vitro (17). An in vivo assay showed that BLID could not only dramatically inhibit breast cancer cell xenograft formation and growth but also inhibit breast cancer cell metastasis in a lung metastasis model. Moreover, it was demonstrated that BLID inhibited breast cancer metastasis. Apoptosis, or programmed cell death (PCD), plays a significant role in normal development and the pathogenesis of cancers (30). Mutated and deleted proapoptotic genes can give rise to carcinogenesis and tumor growth (31,32). Overexpression of BLID caused apoptotic cell death in three different cell lines as evidenced by enhanced chromatin condensation, DNA fragmentation, or an enhanced number of cells in the sub-G_1 phase (15). BLID expression correlated with the activation of caspase-3 and caspase-9. These findings demonstrated that BLID functions as a proapoptotic molecule and suggested that BLID induces a caspase-dependent mitochondrial pathway of cell death. Despite the fact that BLID is defined as a proapoptotic factor, the molecular mechanism by which BLID gene mediates apoptosis and which component is involved in BLID pathway remain largely unknown. In human prostate cancer cells (PC-3), BLID-induced DNA fragmentation was blocked efficiently by coexpression of the antiapoptotic molecule, Bcl-xL (28). An N-terminal deletion mutant of BLID lacking a BH3-like domain or BLID containing a mutant BH3-like domain failed to induce apoptosis, whereas a C-terminal deletion mutant retained the apoptotic activity comparable to the full-length BLID (28).

BLID CONTROLS GENE TRANSCRIPTION

The PI3K/AKT pathway has important roles in the proliferation, migration, and invasion of various cancer types such as gastric cancer, hepatocellular carcinoma, lung cancer, and breast cancer (33–36). Overexpression of BLID significantly inhibited the phosphorylation of AKT Ser 473 and AKT Thr 308, and knockdown of BLID dramatically activated the AKT Ser 473 and Thr 308 (17). Higher expression of MMP-2 and MMP-9 was observed in breast cancer tissues compared with normal samples, associating with both an increased risk of lymph node metastases and poor prognoses (37,38). MMP-2 and MMP-9 can degrade type I and IV collagen and breakdown the extracellular matrix, contributing to cancer metastasis (39). MMP-2 and MMP-9 were found to be significantly decreased in BLID-overexpressing cells, and upregulated in BLID knockdown cells (17) (Fig. 3).

REGULATION OF BLID

As mentioned above, BLID was frequently deregulated in human breast cancer. However, the mechanism leading to its deregulation in human cancer remains unclear. Deregulation of gene expression can be caused by two mechanisms: genetic alterations, namely, gene mutation or loss of heterozygosity (LOH), and epigenetic events, such as CpG island promoter hypermethylation, microRNAs (8,26,40,41).

Inconsistent with studies that show that downregulation of the BLID protein was correlated with younger age (median 40 years), large tumor size, and decreased disease-free and overall survival, all factors of poor prognosis, LOH at two microsatellite markers closely linked and flanking the BLID gene on11q24.1 in 50% of breast tumors from patients with early onset disease (age ≤ 40), compared to 21% and 32% of tumors from patients in the 41- to 55-year-old and $\geq$56-year-old groups, respectively

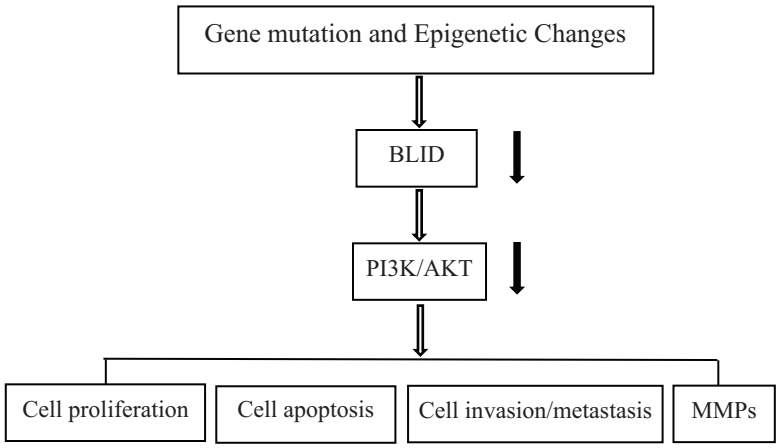

Figure 3. Proposed model of tumorigenesis due to loss or decreased BLID expression. Reduced BLID expression caused by gene mutations or epigenetic changes, suppressed cell apoptosis, induced cell proliferation, metastasis, and angiogenesis by PI3K/AKT pathway.

(27). It suggested that BLID expression may be regulated by LOH in breast cancer.

Doxorubicin, a cytotoxic agent, has been shown to induce the mitochondrial pathway of apoptosis in a variety of cancer cells such as gastric cancer and HeLa cells (42,43). Doxorubicin treatment of cells leads to the activation of BH3-only proteins, Bax, Bak, and caspases. Doxorubicin treatment of HeLa cells led to an increase in the mitochondrial level of endogenous BLID (15). However, the exact molecular mechanism of its deregulation in human cancer remains unclear and needs further investigation.

IMPLICATIONS IN CANCER MANAGEMENT

Given that deregulated expression of BLID was identified in many human cancers, it provides an attractive approach for anticancer therapies. First of all, BLID can be used as a biomarker for cancer diagnosis. By monitoring the BLID status in an individual tumor, such as alterations in gene and protein levels, we can predict the risk of cancer development and progression, as well as the prognosis of the cancer. Since BLID was downregulated in breast cancer, and BLID could inhibit cell growth and metastasis in breast cancer cells in vitro, reintroduction of BLID gene or to stabilization of BLID protein level may be an attractive strategy for cancer management.

CONCLUSIONS AND FUTURE DIRECTIONS

In conclusion, BLID, mutated or depleted in a variety of human malignances, is a well-characterized tumor suppressor. BLID acts as tumor-suppressor function through induction of apoptosis and regulation of gene transcription. BLID can regulate the expression of various tumor-related genes and proteins, such as AKT and MMP, all of which are involved in the tumorigenesis. However, studies on the molecular mechanism of the deregulations of BLID are still limited and needed further investigation.

Previous studies have only focused on the tumor-suppressing role of BLID in breast cancer. However, the expression and function of BLID in other tumors remains unclear. In addition, relatively little is known about the mechanism leading to downregulation of BLID in human cancers. Genetic and epigenetic susceptibility as well as environmental factors might play a role in this process. A better understanding of the upstream regulator for BLID could provide important insights to design better strategies to restore BLID tumor-suppressor function, which might be a potential efficient means to treat cancer patients.

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