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REVIEW ARTICLE

The Wnt-dependent and Wnt-independent functions of BCL9 in development, tumorigenesis, and immunity: Implications in therapeutic opportunities

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Abstract B-cell CLL/lymphoma 9 (BCL9) is considered a key developmental regulator and a well-established oncogenic driver in multiple cancer types, mainly through potentiating the Wnt/β-catenin signaling. However, increasing evidences indicate that BCL9 also plays multiple Wnt-independent roles. Herein, we summarized the updates of the canonical and non-canonical functions of BCL9 in cellular, physiological, or pathological processes. Moreover, we also concluded that the targeted inhibitors disrupt the interaction of β-catenin with BCL9 reported recently.

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Introduction

Human *B-cell CLL/lymphoma 9* (*BCL9*) was first cloned from translocation t(1; 14)(q21; q32) of a patient with precursor B-cell acute lymphoblastic leukemia (ALL) in 1998.[1] Increasing evidences have demonstrated the role of BCL9 in various diseases, especially in various human cancers (Table 1). BCL9 is overexpressed in multiple kinds of cancers, such as myeloma,[2] colorectal cancer (CRC),[3] breast cancer,[4] lung cancer,[5] liver cancer,[6] and ovarian cancer.[7] As an oncoprotein, BCL9 promotes cancer development mainly through sustaining cancer cell division,[8] promoting proliferation and migration, inhibiting apoptosis,[7,9] remodeling tumor microenvironment (TME) and immune system, and regulating chromosomal instability and karyotype for tumor evolution.[4,10–12]

Besides tumors, during tooth development, conditional deletion of both *Bcl9* and its homolog *BCL9-like* (*Bcl9L*) in mice produced teeth with defective enamel that was bright white and deficient in iron, which is reminiscent of human tooth enamel pathologies.[13] *BCL9* and *Pygopus* (*PYGO*) perturbations cause developmental heart defects in zebrafish and mice, indicating implicated alterations in BCL9 in human congenital heart defects.[14] In addition, BCL9 mediates the differentiation of myogenic progenitors during muscle regeneration.[15]

BCL9 has long been considered to be involved in the canonical Wnt signaling pathway, but recent studies have revealed diverse roles of BCL9 outside the Wnt signaling pathway.[8,13,16,17] This review summarizes the recent multiple functions of BCL9, which will provide guidance for uncovering the potential roles of this oncogene and for designing more specific and precise inhibitors targeting its multifaceted functions for various human diseases.

The diverse regulations of BCL9 expression

The expression of BCL9 is up-regulated in tumors, and the mechanism is complex. Hypoxia is a common feature of tumors and plays a crucial role in their occurrence and development. In hepatocellular carcinoma (HCC), hypoxia can induce the expression of BCL9, and this is predominantly mediated by hypoxia-inducible factor 1, alpha subunit (HIF1α), and HIF2α.[18] Consistently, in CRC, the ectopic expression of HIF1α, but not HIF2α up-regulates the expression of BCL9 in SW480 and HCT116 cells at the transcription level, and there is a strong correlation between the expression of HIF1α and BCL9 in human CRC specimens.[19]

Noncoding RNAs are also important regulators of BCL9 (Table 2). In CRC, the expression of miR-140-3p in patients is lower than that in healthy controls, just as in CRC patients with liver metastasis. Notably, BCL9 and BCL2 are identified as direct targets of miR-140-3p.[20] In gastric cancer, the double-stranded miR-30a precursor produces two single-stranded and mature miRNAs: miR-30a-3p and miR-30a-5p. They serve significant biological functions in two distinct ways. Through BCL9, miR-30a-3p inhibits COX-2 expression and regulates β-catenin nuclear translocation. miR-30a-5p binds to the 3′ UTR of BCL9 and negatively regulates BCL9 expression.[21] It is further identified that nuclear paraspeckle assembly transcript 1 (NEAT1), a long non-coding RNA (lncRNA), can sponge miR-30a. NEAT1 is negatively correlated with miR-30a (miR-30a-3p and miR-30a-5p). Because miR-30a-5p negatively regulates the expression of BCL9, NEAT1 enhances the expression of BCL9 indirectly.[22] Also, miR-30c-2* stops growth factor-induced cellular proliferation in ovarian cancer and down-regulates the activity of the oncogene BCL9.[23] In HCC, miR-1301

Table 1 Multi-functions of BCL9.

	Functions of BCL9	References
Cancer development	Promoting cell proliferation, migration; suppressing apoptosis; remodeling tumor microenvironment	1,4,5,7,8,11,56,89
Cancer immunity	Suppressing Treg and DCs; inhibiting the infiltration of $CD8^+$ T cells	4,10
Muscle regeneration	Promoting myogenic progenitors	15
Tooth development	Promoting enamel formation	13
Congenital heart defects	Promoting heart development	14

Table 2 The noncoding RNAs that regulate the expression of BCL9.

Noncoding RNAs	Kinds of cancer	BCL9 expression regulation	References
miR-1301	Hepatocellular carcinoma	Downregulated	24
miR-140-3p	Colorectal cancer	Downregulated	20
miR-30-5p	Myeloma	Downregulated	26
miR-30a-5p	Gastric cancer	Downregulated	21
miR-30c	Prostate cancer	Downregulated	25
miR-30c-2*	Ovarian cancer	Downregulated	23
NEAT1	Gastric cancer	Upregulated	22

inhibits cell migration, invasion, and angiogenesis by decreasing Wnt/β-catenin signaling through targeting BCL9.[24] In prostate cancer, miR-30c functions as an important suppressor of BCL9, and the decreased expression of miR-30c is correlated with a frequent pathogenetic event.[25] In multiple myeloma, the interaction of tumor cells and bone marrow stromal cells leads to the down-regulation of miR-30-5p, thereby enhancing the expression of BCL9.[26]

Recent studies have illustrated that the expression of BCL family members is also regulated by the deregulation of alternative splicing.[27–29] As BCL9 has several splice variants, suggesting the expression of these splice variants may also be regulated by alternative splicing, while additional researches are required. Hypoxia, coding and non-coding factors, and combing targeting BCL9 are among the several elements that regulate the expression of BCL9, and these regulators may be an effective approach for treating various cancers.

The canonical role of BCL9: A nuclear coactivator for Wnt/β-catenin signaling

In 2002, *legless* (*lgs*), the homolog of BCL9 in drosophila was identified. The deletion of *lgs* displayed similar phenotypes to that of Wnt factor Wingless,[30] indicating a potential regulatory role of BCL9 in Wnt signaling. Wnt signaling pathways are highly conserved across animal species, from the fruit fly to human, and governing various fundamental biological processes during development. The aberrant activation of Wnt signaling has been frequently observed in many diseases, especially in cancer.[31–36]

The canonical Wnt pathway was discovered in mouse mammary tumor virus (MMTV) in 1982.[37] Canonical Wnt signaling is stimulated by Wnt ligands that bind to Frizzled (Fz)/low-density lipoprotein (LDL) receptor-related protein (LRP) receptors and activates β-catenin (or *Armadillo* in *Drosophila*) by blocking its phosphorylation and degradation, promoting β-catenin translation from the cytoplasm into the nucleus.[38–40] Through proteasome, β-catenin/*Armadillo* is rapidly degraded in the cytoplasm in the absence of Wnt stimulation by phosphorylation of its amino terminus by the concerted action of the scaffold protein Axin, glycogen synthase kinase 3β (GSK3) and the adenomatous polyposis coli (APC).[41] To transcribe Wnt target genes in the nucleus, β-catenin binds to T cell factor/lymphocyte enhancer factor (TCF/LEF) transcription factors and recruits chromatin-modifying and -remodeling complexes.[42] PYGO and BCL9 (Lgs in *Drosophila*) are conserved nuclear proteins regulating the transcriptional activity of β-catenin/Armadillo.[30,41] PYGO was identified in *Drosophila* as a dedicated component of the Wg (fly homolog of mammalian Wnt). There are two PYGO proteins in mammals, PYGO1 and PYGO2. They have two distinct conserved domains, an N-terminal homology domain (NHD) and a C-terminal PHD zinc finger motif.[41,43,44] PYGO forms a chromatin reader by binding to the N-terminal domain of β-catenin via BCL9/Lgs.[30] As a result, BCL9 has been identified as an adapter and essential core component of nuclear β-catenin complexes,[30,45] and functions as an adapter protein providing binding sites for the transcriptional machinery of Wnt signaling.[42,45,46] This is the canonical function of BCL9.

The primary structure of β-catenin is composed of N-terminal and C-terminal tails flanking a central domain of about 500 residues composed of 12 armadillo (Arm) repeats, which can be packed into a superhelix with a positively charged groove.[47,48] The Arm repeat domain mediates the binding of β-catenin with APC, Axin, Cadherins, TCFs, as well as BCL9 (Fig. 1A).[13,30,49] BCL9 constitutes 5 homeodomains, named HD1～HD5 from its N-terminal to C-terminal. As a reported adapter, the HD1 domain of BCL9 binds the PHD domain of PYGO and the HD2 domain mainly interacts with β-catenin,[30] to activate downstream transcription or shuttle β-catenin in or out of the nucleus in different models (Fig. 1B).[42,50] Thus, BCL9, β-catenin, and PYGO can form a critical nuclear complex for TCF1/LEF1-directed Wnt transcriptional activity and cancer cell growth. Importantly, the regulatory factors or small molecular compounds disrupting the BCL9 and β-catenin interaction can inhibit cancer cell growth.

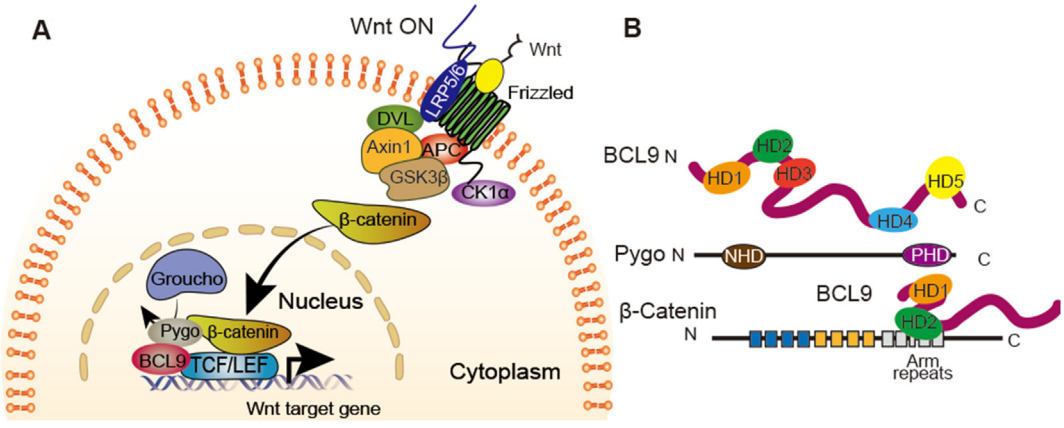

Figure 1 BCL9 functions as a Wnt coactivator. **(A)** BCL9 functions as a coactivator of Wnt/β-catenin dependent transcription. BCL9 functions by recruiting the transcriptional coactivator PYGO to the β-catenin/TCF complex, which is required for the transcription of Wnt target genes. **(B)** The specific domains of BCL9 interact with PYGO and β-catenin.

The multifaceted roles of BCL9 independent of canonical Wnt-signaling

Besides the canonical role as a nuclear coactivator for Wnt/β-catenin signaling, evidence has recently shown diverse non-canonical roles of BCL9 in Wnt-independent manners. The dominant-negative mutants of BCL9 depend not only on its β-catenin ligand, but an unknown ligand on its C-terminus,[51,52] and BCL9 itself contains a transcriptional activation domain containing a proline-rich sequence between HD4 and HD5 domains at the C-terminus.[51] Moreover, the gene fusion of BCL9-MEF2D in ALL represents a distinct form of high-risk leukemia. Another gene fusion of the BCL9 HD2 domain to E1A increases the cytopathic effect of an oncolytic adenovirus that targets colon cancer cells, revealing the multi-functions of BCL9 in cancers.[9,53,54] Moreover, BCL9 has multifaceted functions independent of canonical Wnt signaling. This will be addressed in the following.

BCL9 regulates cancer cell mitosis independent of canonical Wnt signaling

Wnt-dependent stabilization of proteins (Wnt/STOP) signaling was introduced by Sergio *et al* in 2014, during clarifying the mechanism of mitotic Wnt signaling.[55] Wnt/STOP signaling is independent of β-catenin, peaks during mitosis, and protects proteins including β-catenin, c-MYC and other GSK3 substrates from degradation.[55] Many mitotic Wnt/STOP targets are crucial for maintaining precise mitotic cell division.[56]

The activation of canonical Wnt signaling is triggered by the Wnt ligands binding to a heterodimeric receptor complex, consisting of a LRP5/6 on the targeted cell surface.[34] Whereas Clathrin-mediated endocytosis (CME) can negatively regulate Wnt signaling through the internalization and degradation of LRP6 receptor complexes.[57] Unexpectedly, our recent work has identified that BCL9 regulates mitotic spindle assembly and precise cell division. BCL9 acts as a positive regulator in mitotic Wnt signaling and stabilizes the LRP6 signalosome by inhibiting Clathrin and CME. Mechanically, BCL9 phosphorylation by cyclin-dependent kinase 1 (CDK1)/Cyclin B1 can compete with Clathrin to bind with LRP6/Axin1 complex and thus dynamically regulate the stability of mitotic Wnt/STOP substrates during cell division (Fig. 2A).[8]

BCL9 and hippo signaling pathway

Hippo signaling is a critical pathway regulating cell proliferation, epithelia—mesenchymal transition (EMT), and apoptosis in cancers.[16] Canonical Hippo signaling inhibits β-catenin/Wnt signaling through a WW domain-containing transcription regulator protein 1, namely TAZ.[58] TAZ suppresses Wnt3A signaling by limiting the ability of CK1ζ/ε to phosphorylate DVL2.[58] Mammalian large tumor suppressors 1 and 2 (LATS1 and LATS2), the homologs of Wts in *Drosophila*, are serine/threonine kinases and key components of the Hippo signaling pathway.[59—62] In the canonical Hippo signaling pathway, LATS2 can inhibit oncogenesis by phosphorylating the transcription factors. Yes 1 associated transcriptional regulator (YAP) and TAZ.

Recently, it has been reported that LATS2 is a nuclear repressor of β-catenin-mediated transcription by disrupting the interaction between β-catenin and BCL9/PYGO2.[16] Moreover, LATS2 also inhibits the recruitment of BCL9 and BCL9L by β-catenin.[16] Previously, we identified a PPPY motif in BCL9 protein sequence,[8] indicating a probable interaction with the WW domain-containing proteins, such as YAP1 (Fig. 2B).[63] More detailed investigations should be performed regarding the precise interactions between BCL9 and Hippo signaling pathway components.

BCL9 interacts with paraspeckle proteins

Based on the recent update of BCL9, it seems that BCL9 is not only involved in transcriptional regulation but dynamically interacts with some cellular components. Paraspeckles are dynamic structures regarded as nuclear condensates or membrane-less organelles that are altered in response to changes in cellular metabolic activity.[64] Recently, it is also reported that BCL9 is recruited adjacent to the interchromosomal regions. It can stabilize the mRNA of calcium signaling and neural genes by interacting with paraspeckle proteins under spontaneous calcium transients or stress conditions (Fig. 2C). This β-catenin-independent function of BCL9 is markedly correlated with a poor-prognosis subtype of colorectal cancer.[17] Moreover, BCL9 also promotes cancer progression through remodeling the TME and sustaining the calcium transients and neurotransmitter-dependent communication in colorectal cancer cells.[17] These pieces of evidences indicate that BCL9 is a multifunctional protein with a temporospatial regulatory manner in cancers.

BCL9 affects the tumor immune microenvironment

Recently, it is found that BCL9-signal transducer and activator of transcription 3 (STAT3) drives transcriptional enhancers to promote ductal carcinoma progression. BCL9 has been identified to regulate both direct targets of STAT3, including integrin β3, COX-2, forkhead box O1 (FOXO1), and p-c-Jun, as well as upstream regulators of STAT3, such as EGFR, IGF, PDGF, HER2, ERK/MAPK, HGF, ILK, and IL-6.[65] Moreover, knocking down of BCL9 is associated with the upregulation of a subset of tumor suppressors, including BCL2-associated agonist of cell death (BAD), cyclin-dependent kinase inhibitor 1B (CDKN1B), and phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase (PTEN).[65] STAT3 is a 92 kDa protein activated as a DNA-binding protein through the signaling primed by cytokines, such as IL-6 and growth factors (*e.g.*, EGF). The canonical pathway of STAT3 signaling depends on the Y705 phosphorylation,[66] whereas the non-canonical pathway of STAT3 signaling has been implicated in undergoing phosphorylation at the serine 727 (S727) residue.[10] STAT3 is phosphorylated at S727 via the MAPK signaling pathway. The S727 residue is located at a conserved Pro-X-Ser-Pro sequence, recognized by the protein kinase ERK. Activation through the S727 residue is thought to cause transcription initiation.[64] Recently, it has been revealed that BCL9 and pS-727-STAT3 rather than pY705-STAT3 form a complex to enhance the transcription of subsequent genes. Moreover, integrin β3 and its associated matrix

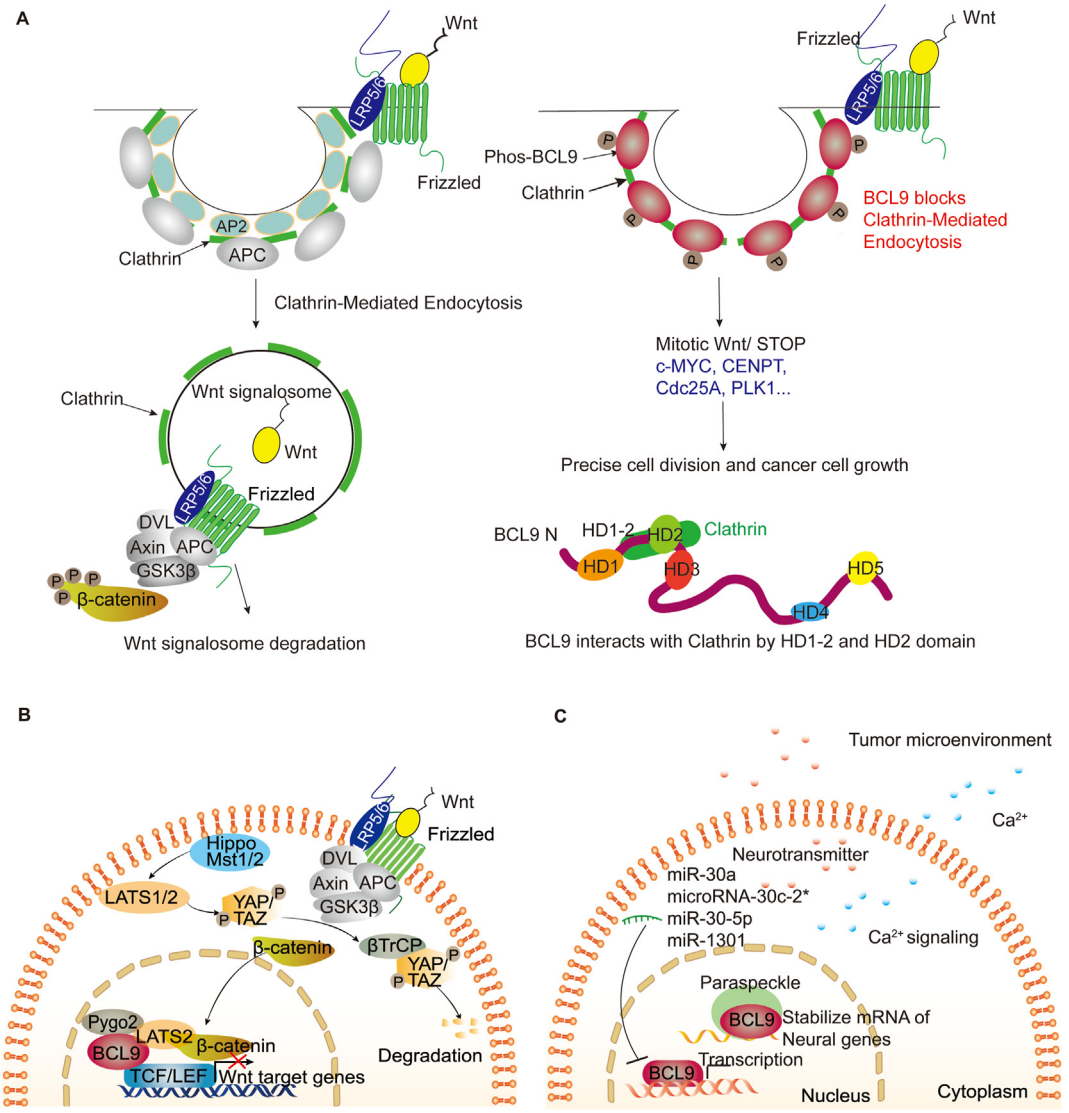

Figure 2 The diverse mechanisms of BCL9 independent of canonical Wnt-signaling. **(A)** BCL9 competes with Clathrin to promote mitotic Wnt/STOP. **(B)** BCL9 and Hippo signaling pathway. **(C)** BCL9 interacts with paraspeckle proteins and microRNAs.

metalloproteinase-16 (MMP16) function as the linker of BCL9/STAT3-associated enhancers to promote human ductal carcinoma *in situ* invasive progression.[65] This finding indicates the interaction of BCL9 and pS727-STAT3, suggesting that BCL9 may be involved in STAT3-mediated immuno-regulation in cancer progression (Fig. 3A).

Interestingly, BCL9 also affects tumor immune infiltration (Fig. 3B). In colon adenocarcinoma, *Bcl9*-depletion combined with PD-1 blockade remodels the tumor immune microenvironment. In *Bcl-9*-depleted CT26 tumors, pharmacological inhibition of *BCL9* enhances cytotoxic $CD8^+$ T and NK&T cell infiltration and inhibits the regulatory T cells (Tregs) migration.[4]

CD155 is an onco-immunological protein identified as a poliovirus receptor (PVR). It is a very important part of how tumor cells invade and move.[15,67] Both CD226 and CD96 are receptors for CD155. CD226 is a transmembrane receptor expressed on $CD8^+$ T cells and NK cells,[11] whereas CD96 is a coinhibitory receptor on NK cells. CD96 not only competes with CD226 for binding with CD155 but also directly inhibits NK cell function.[2] Mechanically, BCL9 potentially modulates the CD226-CD155 checkpoint in $CD8^+$ T cells.[4] The expression of BCL9 is positively correlated with the expression of CD155 in cancer cells and negatively correlated with the expression of CD226 in T cells. BCL9 inhibition induces the engagement of CD226; this activates the proto-oncogene vav (VAV1) and promotes CD155 expression.[4] Moreover, BCL9 depletion also decreases C—C motif chemokine 22 (CCL22) expression in tumor cells. Pharmacological inhibition of BCL9 leads to C—C motif chemokine receptor type 4 (CCR4) expression and decreased Treg infiltration.[4] The peptide "LEHRERSLQTLRDIQRMLFP-Ahx-C" copolymerized with gold ion and assembled into a nanohybrid cluster inhibits tumor growth and metastasis by blocking the interaction of BCL9 with β-catenin. It also synergizes with the PD1/PD-L1 checkpoint blockade immunotherapy,[68] indicating that BCL9 is involved in the modulation of TME.

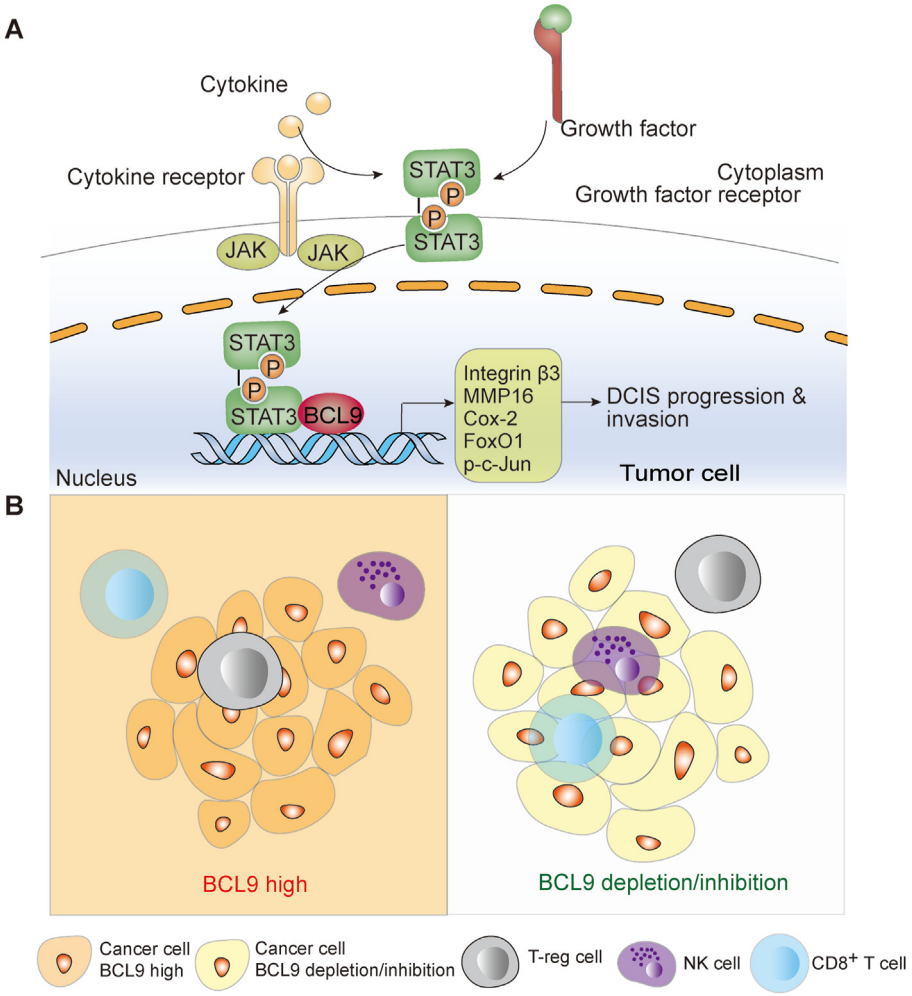

Figure 3 BCL9 is involved in the modulation of the tumor microenvironment. **(A)** The BCL9-STAT3 axis drives cancer progression and invasion. **(B)** BCL9 regulates immune cell infiltration in tumor cells.

The role of BLC9 in regulating TME suggests that it can be a potential target for immunotherapy to help overcome the current obstacles of immunotherapy in the clinic. Though immunotherapy that blocks immune checkpoints has helped many cancer patients, only a small percentage of those who have received it have had a long-term response. Why some patients fail or do not sustain a response to immunotherapy that blocks immune checkpoints is an intense research topic. Immunotherapy resistance mechanisms are classified as either primary or acquired.[69] The primary resistance indicates the patients do not initially respond to the treatment of blocking immune checkpoint; the acquired resistance indicates the patients respond initially but become refractory.[69] An important indicator of the response to immunotherapy is the infiltration of cytotoxic T cells into the TME, which has been demonstrated in ovarian cancer, colorectal cancer, and melanoma.[70–72] This potential mechanism of resistance to immunotherapy suggests that therapy directed at blocking immune checkpoints might synergize with other treatments that enhance endogenous antitumor immunity.

The summarized evidences above have elucidated a potential role of BCL9 in the TME, and depletion of BCL9 could enhance the infiltration of NK cells and T cells.[4] Blocking BCL9 interaction with other interactors may disrupt the resistance to immunotherapy induced by blocking immune checkpoints and improve the efficiency of immunotherapy. These mechanisms need further investigation.

BCL9 and its homolog BCL9L work redundantly or not?

BCL9 has gained variable faces by gradually exploring its canonical and non-canonical roles in human cancer and other diseases. A natural question raised is how about BCL9L protein? BCL9L is also known as BCL9-2, located at chromosome 11. BCL9 and BCL9L share three conserved domains, HD1–3.[73,74] In the nucleus, BCL9 and BCL9L act as the nuclear coactivators of β-catenin and adapters for binding PYGO to prime β-catenin transcriptional

signaling.[73,75,76] Intriguingly, in ameloblasts, BCL9 and BCL9L possess a cytoplasmic function by binding with PYGO to interact with secretory vesicles and modulate the secretion and maturation of the enamel matrix.[13,77]

However, increasing experimental evidences indicate that the expression of BCL9L is mainly responsive to the changes in Wnt pathway activity.[51] The nucleus localization of BCL9L is mainly dependent on its nuclear localization sequence (NLS) at the N-terminus (amino acids 1—175),[74] while BCL9/legless lack the NLS and can localize to both the nucleus and cytoplasm.[7,74] The subcellular localization of BCL9 may depend on its binding with PYGO.[42,78] Whether BCL9 and BCL9L function redundantly in humans by compensating each other in the β-catenin transcriptional complex remains obscure. Silencing of BCL9L significantly affects β-catenin/Wnt signaling, while the silencing of BCL9 does not.[79] The findings of these studies suggest that BCL9 and BCL9L do not work redundantly, at least in the regulation of β-catenin/Wnt signaling. Our previous report has confirmed that BCL9 mainly localizes on the mitotic centrosomes and spindles during mitosis, while BCL9L was detected without this localization of the mitotic apparatus.[8] The diverse, endogenous subcellular localization of BCL9 and BCL9L indicates that BCL9L, rather than BCL9, mainly potentiates the Wnt-induced transcription in a cell or tissue-dependent manner.[51] The cytoplasmic, mitotic, and subcellular detailed distribution of BCL9 in cells may contribute to its non-canonical functions distinct from BCL9L.

Targeting the β-catenin/BCL9 complex

The diverse roles of BCL9 in developmental diseases and cancers suggest BCL9 has great potential as a drug target for clinical application. BCL9 is a long spaghetti-like unstructured protein with five HD domains (Fig. 4). The crystallographic and chemical analysis has identified that BCL9 and its paralogue BCL9L interact with the first armadillo repeat of β-catenin through HD2.[45] Based on the structure, a few compounds have been screened or designed, including the small molecules,[80] stapled helices mimicking the HD2 domain,[81] and some natural compounds[82] for blocking the binding of BCL9/BCL9L with β-catenin (Fig. 4). The small molecule 1,4-dibenzoylpiperazine can block the interaction of β-catenin/BCL9 in colorectal cancer cell lines SW480 and HCT116.[80] Furthermore, carnosic acid (CA) is a natural compound extracted from rosemary. In colorectal cancer cells with hyperactive β-catenin signaling, CA can significantly inhibit BCL9/β-catenin binding. Evidences from nuclear magnetic resonance (NMR) and analytical ultracentrifugation have demonstrated that the CA response requires an intrinsically labile α-helix (H1) amino-terminally abutting the BCL9-binding site in β-catenin.[82] Moreover, therapeutic targeting of BCL9 by CA can inhibit the invasive progression of human ductal carcinoma *in situ*.[65] Nowadays, some other natural compounds that have been proven to be associated with β-catenin signaling may also interact with BCL9/β-catenin.[83—86]

Besides natural compounds, peptides targeting β-catenin/BCL9 have also been designed. Stabilized α-helix of BCL9 (SAH-BCL9) has been developed to dissociate native β-catenin/BCL9 complex, leading to selective suppression of Wnt targets.[81] Using hydrocarbon-stapled peptide technologies, hsBCL9 CT-24, a peptide disrupting the interaction of β-catenin and BCL9/BCL9L, has been developed.[45] This peptide shows an inhibitory effect on the activity of β-catenin, by suppressing cancer cell growth and promoting the infiltration of cytotoxic T cells by reducing the Tregs.[45]

Moreover, as BCL9/BCL9L paralogs bind with PYGO via the HD1 domain to form a "Wnt enhanceosome",

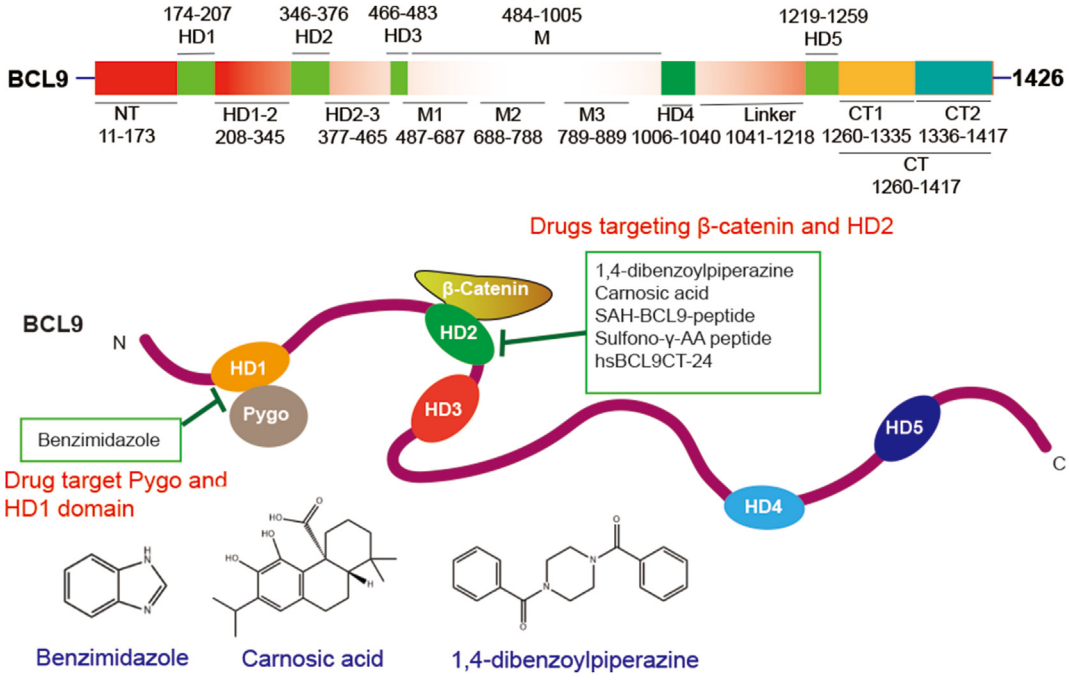

Figure 4 The diagram of BCL9 and the drugs target BCL9.

facilitating β-catenin-mediated Wnt transcription.[87] The HD1 domain of BCL9 assists the PHD finger of PYGO to anchor the histone H3 tail methylated at lysine 4 (H3K4me).[49,88] Benzimidazole docks into the H3K4me specificity pocket and displaces the native H3K4me peptide from the PHD finger, thus blocking BCL9/PYGO mediated chromatin binding.[88]

All the inhibitors mentioned above have exhibited promising effects in preclinical cancer models. Given the diverse functions of BCL9, the designation or development of specific and effective inhibitors of BCL9 remains challenging but necessary. Therefore, the dissection of the regulatory functions and mechanisms of unique domains of BCL9, compared with BCL9L, is critical for developing BCL9 specific inhibitors. Moreover, the combination of the inhibitors targeting BCL9 is rational at least in BCL9-driven cancer. There are still several unanswered questions: (i) What are the regulatory roles of the individual domains of BCL9 in normal cells and cancers? (ii) Is it specifically targetable? (iii) Do BCL9 and BCL9L work redundantly or not in Wnt signaling regulation?

Conclusions

To date, most functional studies on BCL9 are mainly based on its nuclear transcriptional role in the Wnt signaling pathway. However, the investigation of the role of BCL9 in the cytoplasm and specific cellular organelles is crucial for the dissection of its diverse functions and the development of more specific and precise inhibitors in cancer. Previously, we have identified that BCL9 localizes in centrosome and spindle to regulate cell division.[8] However, the detailed domains and modifications that contribute to the localization of the mitotic apparatus require further investigation, which may offer a valuable therapeutic target for the inhibition of cancer cell division. Thus, targeting BCL9 by blocking its interactions with other proteins, such as Clathrin or mitotic spindle proteins would be a promising strategy for the designation of cancer therapy inhibitors.

As BCL9 locates in the centrosome, it remains to be elucidated whether centrosomal BCL9 has elaborate regulation in centrosomal regulation, such as separation, amplification, and cohesion. Furthermore, paraspeckle is a type of membrane-less organelle. Most of the functions of paraspeckle remain obscure. However, it has been proposed that paraspeckles are dynamic structures that are altered in response to the changes in cellular metabolic activity and are transcription-dependent.[89] Does BCL9 play a role in forming paraspeckles? If so, what is the mechanism? Does BCL9 have an organelle-specific function in other organelles or function of the tether of organelle interaction? Too much remains to be elucidated about this multifaceted protein.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

All data generated during this study are included in this published article and the corresponding references.

Author contributions

J.C. contributed to the study design and data analysis and edited the manuscript. M.W., H.D. and C.X. wrote this manuscript. M.S., H.G. and F.B. designed the figures. All authors read and approved the final manuscript.

Conflict of interests

The authors have no competing interests to disclose.

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