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Bruton Tyrosine Kinase (BTK) and Its Role in B-cell Malignancy

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BTK is a kinase that functions downstream of multiple receptors in various hematologic cells. This review focuses on BTK-dependent pathways that are likely to be involved in maintaining the malignant phenotype in B-cell lymphomas and leukemias. Survival of various B-cell malignancies requires BTK-dependent signals from the B-cell antigen receptor. Survival is also dependent on malignant cells homing to and interacting with lymphoid microenvironments, and these interactions are also BTK-dependent due its role in signaling downstream of chemokine and innate immune receptors. The potential for therapeutic targeting of BTK is currently being tested in clinical settings.

Keywords B-cell receptor, kinase, leukemia, lymphoma, PCI-32765, X-linked agammaglobulinemia

The discovery and naming of Bruton tyrosine kinase (BTK) derives from the 1952 description of the rare X-linked immunodeficiency (XLA) syndrome by Ogden Bruton, a pediatrician then in the United States Navy [1]. Patients with this condition had frequent severe infections in childhood and were noted to have virtually complete absence of B cells and circulating immunoglobulins. Despite this severe abnormality, patients with this disease are currently able to lead relatively normal lives due to support with gamma-globulin infusions and antibiotics. *BTK*, the gene mutated in this condition and recognized as essential for normal B-cell development, was first cloned and characterized in 1993 [2, 3]. Despite the apparent restriction of clinical features to B-cell immunity in XLA, BTK is actually expressed and functional across all the marrow derived (non-T) hematopoietic lineages [4–6]. The functional tolerance of BTK deficiency in other cell types is apparently attributable to robust redundancy of signaling and homeostatic pathways controlling essential functions. A further surprising aspect is that despite BTK's function in normal B cells, pharmacologic inhibition of BTK does not replicate the clinical picture of XLA; severe manifestations of the genetic deficiency are due to the role of BTK in early differentiation at the stage of pro- to pre-B cells. *Xid* [7] is a Btk functionally deficient murine counterpart of XLA, which is, however, phenotypically less severe than XLA. This difference is thought to be due to greater overlap in function with the kinase TEC in mice than in humans. *Xid* mice have roughly 50% of normal B cells, lack the $CD5^+$ B-1 B-cell subpopulation, and have reduced serum Ig levels [8–10]. B cells isolated from these mice do not proliferate in response to anti-IgM treatment, and proliferation in response to LPS is reduced [11]. In addition, *xid* mice are not able to mount a thymus-independent-type II (TI-II) response to antigens, but do have normal T-dependent immune responses.

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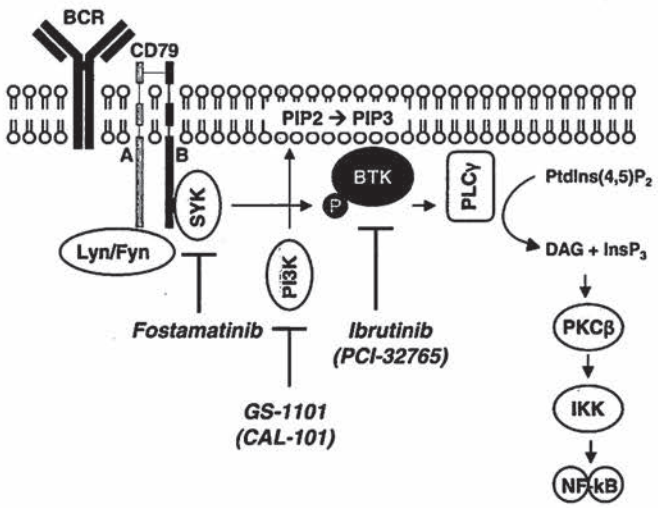

FIGURE 1 A simplified B cell receptor signaling pathway. Upon antigen engagement of the BCR, the co-receptors CD79A and CD79B are phosphorylated by the tyrosine kinases LYN and FYN, which recruits the kinase SYK. Multiple proteins including PI3Kδ, BLNK (not shown) and BTK are recruited to the membrane and form a multiprotein complex sometimes referred to as a signalosome. SYK then phosphorylates multiple substrates including BTK and PI3Kδ. BTK phosphorylates and activates PLCγ, which generates diacylglycerol (DAG) and inositol triphosphate (InsP3), which are necessary for the activation of protein kinase C (PKC). PKC phosphorylates IkB kinase (IKK) and this induces NF-κB activation. The targets of three drugs currently in clinical development (Fostamatinib, PCI-32765, and CAL-101) are shown.

BTK is a member of the TEC family of nonreceptor tyrosine kinases. Its gene encodes a 659 amino acid protein that contains a single kinase domain and multiple protein–protein interaction domains: An NH_2-terminal pleckstrin homology (PH) domain, which binds to phosphatidylinositols during the process of membrane localization, is followed by Src homology 2 (SH2), Src homology 3 (SH3), and proline-rich domains that regulate binding to other cellular signaling molecules [12]. The expression of BTK is typically cytoplasmic, but it is known to translocate to the plasma membrane during the process of B-cell activation [13] through interactions of its PH domain with phosphatidylinositol-3,4,5-triphosphate (PIP3) generated by PI3K (see Figure 1) [4]. Once localized to the membrane, BTK is phosphorylated by SYK or LYN on Y551, and BTK in turn phosphorylates and activates phospholipase-Cγ (PLCγ)[15], leading to Ca^{2+} mobilization [16] and activation of key pathways, including that of mitogen-activated protein kinase (MAPK) [17], as well as activation of the inducible transcription factor NF-κB [18]. The active form also undergoes autophosphorylation at Y223, but this step is of uncertain functional significance. Data from crystal structures of BTK, both alone and complexed with various inhibitors, have revealed specific conformational states associated with the active and inactive forms of the enzyme, and these insights continue to inform the structure-based design of BTK-selective inhibitors [19–21].

The Role of BTK in B-cell Function and Receptor Signaling

The generation and maintenance of B lymphocytes is controlled by biochemical signals transmitted by the B-cell antigen receptor (BCR). The expression of a functional BCR is required for survival during several stages of B-cell development, but also in

mature, resting B cells [22, 23]. Conditional deletion of the BCR in the B cells of mice leads to rapid cell death; this result argues that there is a "tonic" signal that maintains cell viability [24]. The BCR is composed of two identical heavy-chain and light-chain Ig peptides linked by disulfide bridges. The Ig component binds to antigen presented by antigen-presenting cells, but also transmits lower level signals in the absence of antigen engagement [25]. Depending on the developmental stage of the B cell, this signal may trigger B-cell proliferation, survival, or further differentiation. The Ig portion associates with the BCR signaling proteins CD79A (Igα) and CD79B (Igβ), which are subject to phosphorylation on immunoreceptor tyrosine-based activation motifs (ITAMs) by the SRC-family kinases. Phosphorylated ITAMs have extensive scaffolding interactions with adapter molecules and kinases, including SYK kinase, which, along with FYN, is capable of phosphorylating and activating BTK [26, 27]. Genetic experiments in mice have demonstrated that BTK is limiting for the transmission of mitogenic signals from the BCR [10]. LYN is also active in this pathway but may have an inhibitory rather than stimulatory effect on signaling [28, 29].

The BCR activates a variety of different signaling pathways in parallel [18]. The specific signaling pathway responsible for maintaining normal B-cell survival appears to require PI3K [30], with downstream signaling by BTK being critical to regulation of apoptosis by NF-κB [31]. Direct BTK coupling of BCR signaling to NF-κB is evident from the lack of its activation by BCR in the *xid* model [32]. Activation of NF-κB alone does not, however, rescue the *xid* phenotype, suggesting that NF-κB is not the sole survival signal in normal B cells. BTK activates NF-κB by phosphorylating PKCβ, which in turn phosphorylates IKK [33], resulting in its dissociation from the NF-κB complex, allowing it to undergo nuclear translocation and function as a transcription factor. Recent evidence suggests that BCR signaling may be amplified by a positive feedback loop involving the B-activating factor (BAFF) receptor BR3 [34]. This pathway can be activated by genes under control of the c-rel/p65 subunit of NF-κB, which is capable of augmenting BTK transcription [35].

While BTK's role in signal transduction is best known as a key signaling molecule for the BCR, it is also functional in other receptor pathways (Table 1), including immunoglobulin Fc receptor signaling [36–38], and the G-protein coupled chemokine receptors CXCR4 and CXCR5, essential for B-cell trafficking and tissue homing [39–41]. Lymphocyte homing to and adhesion within peripheral lymph nodes and secondary lymphoid tissues are regulated by a complex molecular signaling pathway whereby chemokine binding to their receptors leads to the "inside-out" activation of cell surface integrin proteins [42]. BTK activation is thought to occur following a direct interaction with the chemokine receptor G protein subunits [43, 44], with BTK then mediating chemokine-controlled migration through PLCγ2. The BCR itself has been shown to regulate integrin $\alpha4\beta1$ (VLA-4)-mediated adhesion to cellular substrates such as vascular cell adhesion molecule-1 (VCAM-1) and to fibronectin, and genetic knockdown of BTK inhibited this integrin function [45]. BTK has been shown to interact with several members of the Toll-like receptor (TLR) family, including TLR8 and TLR9 [46–48]. Stimulation of B cells with the TLR9 agonist CpG activates BTK and results in cellular proliferation, cytokine production, and activation of NF-κB; each of these effects are blocked in BTK-negative B cells [49, 50]. Most B-cell malignancies respond to CpG oligonucelotides by increasing proliferation and the expression of co-stimulatory and antigen-presenting molecules [51, 52].

While the canonical BCR activation pathway has been most widely investigated, it should be noted that a large number of other signaling and scaffolding interactions of BTK have been reported but less widely studied, including the Wnt/β-catenin [53] and JNK/SAPK pathways [54], the IL-6 signaling molecule gp160 [55], and the transcription factor Bright [56]. Thus, the complexity of BTK functioning is far from fully elucidated.

TABLE 1 Cell surface receptors that signal through Btk

Receptor	Ligand	Cell type(s)	Biological process	Reference
B cell antigen receptor (BCR)	Antigens	B cells	Proliferation and differentiation	[22]
BAFF receptor (BR3)	B cell activating factor (BAFF)	B cells	B cell survival, self tolerance	[34]
CXCR4 and CXCR5	SDF-1 (CXCL12), CXCL13	B cells	Migration and homing	[40]
FcγR	Immune complex	B cells, monocytes, macrophages, neutrophils, dendritic cells	Immune activation, cytokine secretion	[38], [128], [129]
FcεRI	IgE	Mast cells, basophils	Degranulation, cytokine secretion	[66], [67], [68], [69], [103]
Glycoprotein Ib, VI	Von Willebrand Factor (VWF), Collagen	Platelets	Aggregation, agglutination	[72], [73], [74]
Receptor activator of nuclear factor-κB (RANK)	RANK ligand (RANKL)	Osteoclasts	Osteoclastogenesis	[62], [63]
Toll-like receptors 4, 8, 9 (TLR 4, 8, 9)	Lipopolysaccharide, single stranded RNA, microbial nucleic acids	B cells, macrophages, monocytes, dendritic cells	Cytokine secretion, immune activation	[47], [48], [49], [50], [130]

BTK in Non-lymphoid Lineages

While the defect in B-cell development is the most salient feature of *xid*/XLA, abnormalities in other lineages are detectable. These appear to be of limited in vivo functional significance, presumably related to physiologic redundancies, including utilization of TEC as an alternative signaling molecule within specific cells as well as overlapping cellular functions. BTK has thus been demonstrated to participate in the regulation of multiple inflammatory effector functions, including nitric oxide induction, cytokine secretion, and bactericidal functions in macrophages [57] and neutrophilic chemotaxis, transmigration, adhesion, and maturation [58, 59]. BTK was shown to be activated by the hypoxia-induced mitogenic factor (HIMF) receptor, which stimulates bone marrow myeloid cell migration [60]. BTK was also shown to regulate E-selectin-mediated integrin activation and recruitment of neutrophils [58]. Polymorphonuclear neutrophil granulocytes (PMNs) from *xid* mice were shown to have impaired recruitment to sites of inflammation [61], and treating mice with collagen-induced arthritis with the BTK inhibitor PCI-32765 resulted in near complete clearance of inflammatory infiltrates of affected joints [38].

BTK is expressed in osteoclasts (OC) and is important in their function and development from monocytes. Although neither *Xid* mice nor XLA patients have bony abnormalities, monocytic OC precursor cells from *Xid* mice are defective in multinucleate osteoclast formation in response to RANKL [62]. Btk/Tec knockout (KO)mice (but not Btk KO mice)have osteopetrotic bones associated with defective osteoclast formation [63]. XLA patients have a similar defect in OC formation detectable in vitro, which may be compensated for in vivo by higher levels of regulatory cytokines [64], thus masking clinical manifestations.

In mast cells and basophils, BTK lies downstream of the high-affinity IgE receptor (FcεRI), and mediates signaling events that control the process of degranulation [65–68]. Btk knockout mice are known to have impaired mast cell degranulation following FcεRI cross-linking [69]. Mast cells may contribute to a microenvironment favorable for progression of a number of solid tumors and early evidence suggest that BTK could be a therapeutic target in such settings [70, 71]. BTK is expressed in platelets, and has been reported to be involved in signaling from both the GPVI collagen receptor and GPIV von Willebrand complex receptor, both of which signal through Fc$R\gamma$ and FYN, LYN, and SYK. In vitro impairment of collagen and shear stress-induced aggregation due to deficient BTK activity have been reported, although no evidence has been noted of a clinical bleeding diathesis among XLA patients [72–74].

Expression of BTK appears to be strongly restricted to hematopoietic lineages with the sole possible exception being reported expression by some colon tumors and cell lines [53]. A number of studies have indicated an increased incidence of colon cancer in XLA patients [75, 76], but this is the only example reported of increased cancer risk in these patients. While evidence has been presented of a capability of BTK to act as a tumor suppressor [77], and an inhibitor or wnt β-catenin signaling [53], a relationship, as suggested by Mohamed et al. [78], to altered intestinal flora and local inflammation due to deficient IgA might be a more likely basis for this clinical association.

Targeting BTK in Cancer

B-cell Lymphoproliferative Diseases

There is a broad body of evidence pointing to an essential role of BCR and chemokine receptor signaling in the most common types of B-cell lymphoproliferative cancers, providing a general rationale for targeting BTK for treatment of these conditions. Normal B cells are under selective pressure to maintain expression of an appropriate

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