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COMMENTARY

Bruton's Tyrosine Kinase (BTK) as a Dual-Function Regulator of Apoptosis

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ABSTRACT. Multiple counterregulatory mechanisms have been identified in B-cell precursors that operate to regulate cell survival and growth, thereby ensuring the orderly development and differentiation of B-cells. Inappropriate apoptosis may underlie the pathogenesis of immunodeficiencies, as well as pathogenesis and drug/radiation resistance of human leukemias and lymphomas, which makes control of apoptosis an important potential target for therapeutic interventions. Therefore, identification of the molecular regulators of apoptosis is an area of intense investigation. Bruton's tyrosine kinase (BTK) is the first tyrosine kinase to be identified as a dual-function regulator of apoptosis, which promotes radiation-induced apoptosis but inhibits Fas-activated apoptosis in B-cells. BTK functions in a pro-apoptotic manner when B-cells are exposed to reactive oxygen intermediates, at least in part, by down-regulating the anti-apoptotic activity of STAT-3 transcription factor. In contrast, BTK associates with the death receptor Fas and impairs its interaction with Fas-associated protein with death domain (FADD), which is essential for the recruitment and activation of FLICE by Fas during the apoptotic signal, thereby preventing the assembly of a pro-apoptotic death inducing signaling complex (DISC) after Fas-ligation. The identification of BTK as a dual-function regulator of apoptosis will significantly increase our understanding of both the biological processes involved in programmed cell death and the diseases associated with dysregulation of apoptosis. New agents with BTK-modulatory activity may have clinical potential in the treatment of B-cell malignancies (in particular acute lymphoblastic leukemia, the most common form of childhood cancer), as well as B-cell immunodeficiencies. BIOCHEM PHARMACOL **56**;6:683–691, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. apoptosis; tyrosine kinase; signal transduction; immunodeficiency; malignancy; Bruton's tyrosine kinase

APOPTOSIS

Apoptosis is a common mode of eukaryotic cell death, which is triggered by an inducible cascade of biochemical events leading to activation of endonucleases that cleave the nuclear DNA into oligonucleosome-length fragments [1–4]. Several of the biochemical events that contribute to apoptotic cell death, as well as both positive and negative regulators of apoptosis, have been identified recently [5–10]. Apoptosis plays a pivotal role in the development and maintenance of a functional immune system by ensuring the timely self-destruction of autoreactive immature and mature lymphocytes as well as any emerging target neoplastic cells by cytotoxic T cells [11–13]. Inappropriate apoptosis may contribute to the development as well as chemotherapy resistance of human leukemias and lymphomas [11–13]. Therefore, an improved understanding of the molecular basis of apoptosis and the pro-apoptotic versus anti-apoptotic regulatory signals may provide further insights into the pathogenesis of human lymphoid malignancies and have important implications for the treatment of leukemias and lymphomas. Recent studies indicate that BTK‡, a member of the Tec tyrosine kinase family, is a dual-function regulator of apoptosis.

IONIZING RADIATION-INDUCED APOPTOSIS

Apoptosis induced by radiation exposure was originally thought to result from direct interaction between photons (or their resultant charged particles) and DNA, thereby causing breakage of chromosomes. When it was found that ROI were the major culprit in radiation-induced damage the common hypothesis became that DNA damage conferred by ROI was responsible for the induction of apoptosis. Supporting evidence was that radiation-induced ROI

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‡ *Abbreviations:* BTK, Bruton's tyrosine kinase; DISC, death inducing signaling complex; FADD, Fas associated protein with death domain; GAS, gamma-activated sequence; IFN, interferon; ISRE, IFN-α-stimulated response elements; PH, pleckstrin homology; PTK, protein tyrosine kinase; PV, pervanadate; RIP, receptor-interacting protein; ROI, reactive oxygen intermediate(s); SH, Src homology; STAT, Signal Transducers and Activators of Transcription; TNF, tumor necrosis factor; and TRADD, TNF receptor-1-associated death domain protein.

were found to cause single- or double-strand breaks in DNA, a number of types of base alterations, or DNA-protein cross-links [14]. Whereas very high energy photons or particle beams with high linear energy transfer potential produce large numbers of double-strand DNA breaks that, when left unrepaired for extended periods of time, can result in apoptosis, most interactions with photons produce single-strand breaks in DNA that are repaired rapidly and, therefore, are not lethal [15].

Chromosomal DNA is only one of a number of cellular substrates that may be affected by the ROI generated by ionizing radiation. Several published reports have utilized cell-free systems in which isolates of normal nuclei from untreated cells, thus containing intact chromosomes, were combined with cytoplasmic extracts from cells separately exposed to various stimuli, including ionizing radiation, known to provoke apoptosis in intact cells [16–18]. The cytoplasmic extracts from stressed cells invoked changes characteristic of apoptosis in exogenously added nuclei, such as chromatin condensation accompanied by shrinkage and fragmentation of the nuclear envelope that was coincident with internucleosomal cleavage of DNA, whereas cytoplasm extracts obtained from untreated cells induced none of these changes. Therefore, it became apparent that components capable of activating the apoptotic pathway are present in the cytoplasm and operate on previously undamaged nuclear elements, including DNA. In a separate study, enucleated cells subjected to oxidant-induced stress showed morphologic changes typical of apoptosis, including whole-cell shrinkage and blebbing of the cell membrane into "apoptotic bodies," thereby confirming that the nucleus is not essential for the initiation of apoptosis [19].

A long-standing puzzle in radiobiology has been what biochemical or biophysical signals are responsible for initiating radiation-induced apoptosis. Several apoptotic signals, including those induced by TNF, taxol, engagement of antigen receptors on T- and B-cells, ceramide, and dexamethasone, are now known to be triggered by the activation of PTKs. Similarly, activation of PTK has been implicated in radiation-induced apoptosis [20, 21]. Reactive oxygen intermediates, hydroxyl radicals in particular, have been reported as mediators of radiation-induced activation of PTK [22].

The biophysical mechanism underlying the radiation-induced activation of the PTK has not been fully deciphered [22, 23]. A plausible explanation is provided by Pu *et al.* in a recent report [24]. This study showed that an ROI mimic (Hg^{2+}) increased by several-fold the catalytic activity of a purified Src-family PTK. The activity of the sulfhydryl-reactive Hg^{2+} ion was shown to emulate the activity of ROI because it could be neutralized by the sulfhydryl-containing, radical scavenger molecule *N*-acetylcysteine. Direct interaction of Hg^{2+} with sulfhydryl residues on the PTK promoted autophosphorylation on a tyrosine residue, with marked elevation of kinase activity, even on kinase molecules that remained phosphorylated at the negative regulatory site(s). A similar mechanism may account for the ability of ROI generated by ionizing radiation to activate PTK.

BTK AS AN INHIBITOR OF THE ANTI-APOPTOTIC STAT-3 ACTIVATION SIGNAL GENERATED IN RESPONSE TO REACTIVE OXYGEN INTERMEDIATES

BTK is a cytoplasmic PTK involved in signal transduction pathways regulating growth and differentiation of B-lineage lymphoid cells [25, 26]. Mutations in the human *btk* gene are the cause of X-linked agammaglobulinemia (XLA), a male immune deficiency disorder characterized by a lack of mature, immunoglobulin producing, peripheral B-cells [27, 28]. BTK is a member of the BTK/Tec family of PTKs [25, 26], distinguished by an N-terminal region consisting of a PH domain followed by a proline-rich Tec homology (TH) domain. The PH domain is the site of activation by phosphatidylinositol-phosphates and G-protein $\beta\gamma$ subunits, and inhibition by protein kinase C [29]. The remaining portion of BTK contains SH domains SH3, followed by SH2, and a C-terminal SH1 kinase domain. The SH2 domain mediates binding to tyrosine-phosphorylated peptide motifs on other molecules, and the SH3 domain mediates binding to proline-rich motifs. BTK is activated by transphosphorylation of Tyr^{551} in the SH1 domain, followed by autophosphorylation of Tyr^{223} in the SH3 domain [26]. Phosphorylation of Tyr^{223} may function to disrupt an intramolecular TH-SH3 domain interaction, allowing BTK TH domain binding with SH3 domains in the Src family kinases Fyn, Lyn, and Hck, and BTK SH3 domain binding with a proline-rich region of Cbl [26, 30–32]. BTK participates in signal transduction pathways initiated by the binding of a variety of extracellular ligands to their cell surface receptors: following ligation of B-cell antigen receptors (BCR), BTK activation by the concerted actions of the PTKs Lyn and Syk is required for induction of phospholipase C-γ2 mediated calcium mobilization [33].

As discussed earlier, ROI trigger apoptosis in B-cells in a PTK-dependent fashion [20]. A recent study demonstrated that BTK is one of the PTK activated in B-cells exposed to ionizing radiation as well as hydrogen peroxide [34]. B-cells rendered BTK deficient through targeted disruption of the *btk* gene, by homologous recombination knockout, did not undergo radiation-induced apoptosis (Fig. 1), but cells with disrupted *lyn* or *syk* genes did [34]. Introduction of the wild-type, but not a kinase domain mutant, human *btk* gene into BTK-deficient cells restored the apoptotic response to radiation and hydrogen peroxide [34]. Exposure of cells expressing BTK with mutant SH2 or PH domains to ionizing radiation-induced apoptosis, indicating that neither the SH2 domain nor the PH domain of BTK is critical for its pro-apoptotic function (Fig. 2; [34]). These studies provided evidence that BTK is PTK responsible for triggering apoptosis in B-cells exposed to ROI, and its kinase domain is indispensable for its pro-apoptotic function [34].

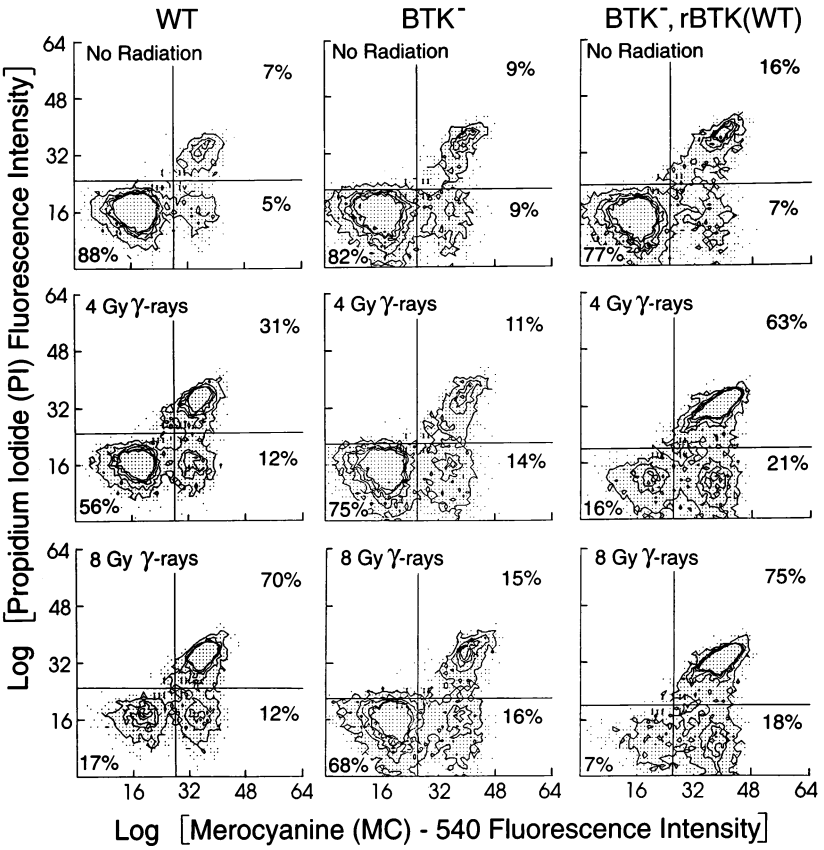

FIG. 1. Effects of BTK in the apoptotic response of B-cells to ionizing radiation. FACS correlated two-parameter displays of wild-type (WT), BTK-deficient (BTK^-) DT-40 cells, and BTK-deficient DT-40 cells reconstituted with the wild-type human *btk* gene [BTK^-,rBTK(WT)] stained with MC540 and PI 8 hr after treatment with PBS, pH 7.4 (no radiation), 4-Gy γ-rays, or 8-Gy γ-rays. The percentages indicate the fraction of cells at an early stage of apoptosis, as measured by single MC540 fluorescence, and the fraction of cells at an advanced stage of apoptosis, as measured by dual MC540/PI fluorescence.

Similarly, BTK has been shown to facilitate apoptosis in murine mast cells deprived of IL-3 [35].

STATs are a family of DNA binding proteins that reside in the cytoplasm until they are activated by tyrosine phosphorylation. They were originally identified in a search for genes transcriptionally induced by IFN-α or -γ in the absence of protein synthesis [36–39]. Analysis of the promoters of these genes led to the identification of recognition sequences called ISRE and GAS in the IFN-γ stimulated genes. Protein purification of the transcription factors that bind these two response elements allowed the cloning of the cDNAs for the first two members of the STAT family: STAT-1 and STAT-2. Four other STAT proteins have since been identified: STAT-3, STAT-4, STAT-5a/b, and STAT-6.

The dual role of STATs as signaling molecules and transcription factors is reflected in their structure. All STAT proteins contain a DNA binding domain, an SH2 domain, and a transactivation domain necessary for transcriptional induction. In unstimulated cells, latent forms of STATs are localized predominantly in the cytoplasm. Ligand binding induces STAT proteins to bind with their SH2 domains to the tyrosine phosphorylated motifs in the intracellular domains of various transmembrane cell surface receptors [40–42]. Once STATs are bound to receptors, the receptor-associated JAK kinases phosphorylate STATs on a single tyrosine residue located near the SH2 domain [43]. Two STATs then dimerize through specific reciprocal SH2-phosphotyrosine interactions [40]. The dimerized STAT proteins can also form complexes with other DNA-binding proteins [36–39]. The STAT dimers/complexes subsequently translocate to the nucleus via an unknown mechanism and utilize their DNA binding domain to interact with DNA response elements in promoters of target genes [44]. STATs then interact directly or indirectly, via their transactivation domain, with as yet unknown components of the RNA polymerase II complex to activate transcription of target genes. Different ligands employ specific JAK and STAT family members; thus, utilization of this pathway mandates specificity in signaling cascades and contributes to a diverse array of cellular responses [36–39, 45].

In addition to activation by tyrosine phosphorylation, the ability of STATs to influence gene transcription is increased by phosphorylation of a C-terminal domain serine residue [46]. SHP-1 protein tyrosine phosphatase inhibits STAT activation at cell surface receptors, as does a recently discovered STAT-induced family of JAK binding proteins that operate in a feedback loop to inhibit activation of STATs [36–39, 47]. Investigations into the termination of STAT signalling have demonstrated that SHP-1 and the ubiquitin/proteasome system function to deactivate and remove STATs from the nucleus [36–39]. Although progress has been rapid, much remains to be understood concerning the structure, function, and regulation of STATs.

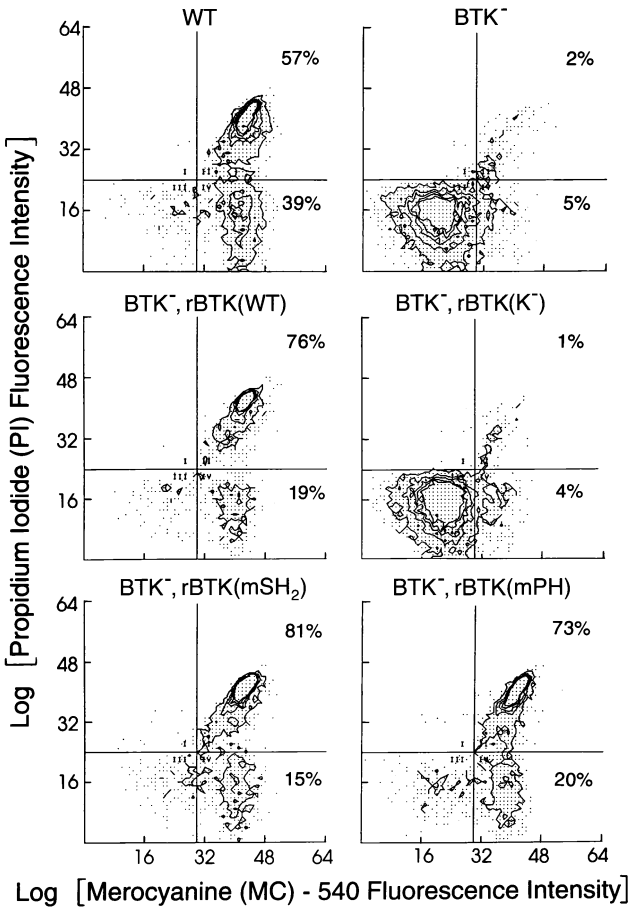

FIG. 2. Domain requirements of the pro-apoptotic function of BTK in irradiated B-cells. Cells were harvested 8 hr after exposure to 8-Gy γ-rays. Unirradiated controls as well as irradiated cells were maintained in culture medium for 8 hr at 37° and 5% CO_2 before harvesting. BTK^- deficient DT-40 (BTK^-) cells expressing wild-type BTK, BTK($Arg^{525} \rightarrow Gln$), BTK($Arg^{28} \rightarrow Cys$), and BTK($Arg^{307} \rightarrow Ala$) were designated as BTK^-,rBTK(WT), BTK^-,rBTK(K^-), BTK^-,rBTK(mPH), and BTK^-,rBTK(mSH_2), respectively. FACS-correlated two-parameter displays of wild-type (WT) and BTK-deficient (BTK^-) DT-40 cells, as well as BTK-deficient DT-40 cells reconstituted with wild-type and mutant human *btk* genes stained with MC540 and PI 8 hr after treatment with 8-Gy γ-rays, are depicted.

Recent studies have correlated STAT activation with signals regulating apoptosis [48–53]. Interleukin-6 (IL-6) is an anti-apoptotic B-cell growth and differentiation factor that promotes the survival of murine B-lineage lymphoid cells in primary cultures and IL-6 transgenic mice, and prevents radiation-induced apoptosis [54–57]. STAT-3 has been shown recently to be activated in cells exposed to ROI [58], and STAT-3 activation was required for the gp130-mediated anti-apoptotic signals triggered by IL-6 and other members of the IL-6 family of cytokines [59]. The pro-apoptotic properties of BTK [34], its association with the gp130 [60, 61], and the reported role of STAT-3 for the anti-apoptotic signals intimately linked to gp130 motivated us to examine the possibility of BTK–STAT-3 interactions under oxidative stress, using PV. We found that STAT-3 is physically associated with BTK in B-cells; this interaction is promoted by PV, and interferes with PV-induced STAT-3 activation.* PV-induced activation of STAT-3 was amplified when cells were rendered BTK deficient by targeted disruption of the *btk* gene, and introduction of a wild-type human *btk* gene into BTK-deficient cells suppressed the PV-induced STAT-3 activation signal observed in the absence of BTK. These results prompted the hypothesis that BTK may facilitate apoptosis in B-cells exposed to reactive oxygen intermediates, in part by interfering with the anti-apoptotic STAT-3 activation signal [57].

The ability of BTK to negatively regulate STAT-3 activity suggests several possible models for a mechanism of BTK action. For example, BTK-mediated phosphorylation and association with STAT-3 may inhibit the ability of STAT-3 to enter the nucleus or bind DNA. This could occur either due to cytoplasmic compartmentalization of STAT-3 or by an inability to dimerize with the relevant STAT partner. Along this line, Chung and co-workers [62] recently identified a protein termed PIAS3 that binds STAT-3 and blocks its DNA binding activity following ligand stimulation. Future investigations will analyze whether PIAS3 association is also stimulated with PV treatment. Phosphorylation of STAT-3 by BTK may also alter the conformation of STAT-3 in such a way as to make it inaccessible as a substrate of activating kinases such as JAK3. Determining the phosphorylation sites of BTK will aid in delineating whether BTK and JAK3 differentially phosphorylate STAT-3. Alternate methods of STAT regulation may also be disrupted by BTK–STAT-3 interactions. A STAT-induced family of JAK binding proteins have been shown to operate in a feedback loop to inhibit activation of STATs [51, 63–65]. Thus, decreasing JAK activation results in decreased STAT activity. However, as BTK does not directly inhibit JAK3 activity, it is unlikely that BTK functions in a manner similar to that of JAB. Phosphorylation of a C-terminal domain serine residue is also needed for STAT activation [47, 66], and SHP-1 protein tyrosine phosphatase inhibits STAT activation at cell surface receptors [67, 68], thus providing other regulatory targets that could be affected by BTK activity.

BTK AS AN ANTI-APOPTOTIC PROTEIN

Mutations in the *btk* gene have been linked to severe developmental blocks in human B-cell ontogeny leading to human XLA [27, 28] and less severe deficiencies in murine B-cells leading to murine X-linked immune deficiency (Xid) [69]. In murine B-cells, BTK has also been shown to act as an anti-apoptotic protein upstream of bcl-xL in the B-cell antigen receptor (but not the CD40 receptor) activation pathway [70].

The Fas/APO-1 (CD95) cell surface receptor, a member

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of the TNF receptor family, is one of the major regulators of apoptosis in a variety of cell types [1–5]. Functional abnormalities of Fas have been associated with pathologic conditions of the immune system homeostasis, including lymphoproliferative disorders, immunodeficiencies, and autoimmunity [11–13]. Identifying the molecules that participate in the apoptotic death signal pathways linked to Fas receptor and finding ways to modulate the activity of such molecules could provide the basis for innovative treatment programs. Ligation of the cell surface Fas molecule rapidly and dramatically induces apoptosis in many but not all Fas positive cell types [12]. DT-40 is a chicken lymphoma B-cell line that we have used previously to elucidate the molecular mechanism of radiation-induced apoptosis [34]. Despite their abundant surface expression of Fas, DT-40 cells are very resistant to the cytotoxic effects of Fas-ligation, indicating the existence of potent negative regulators of Fas-mediated apoptosis. In a series of experiments designed to examine the potential negative regulatory role of BTK in Fas-mediated apoptosis, we found that less than 10% of wild-type DT-40 cells treated with the anti-Fas antibody showed apoptotic changes, whereas virtually 100% of BTK-deficient DT-40 cells underwent apoptosis.* Notably, BTK-deficient DT-40 cells reconstituted with a wild-type human *btk* gene displayed very little evidence of apoptosis, which provided formal proof that BTK plays a pivotal role in preventing the apoptotic death signal triggered by Fas ligation. Fas activation of reconstituted BTK-deficient DT-40 cells expressing human BTK with mutations in the kinase, SH2, or PH domains induced apoptosis as in non-reconstituted BTK-deficient DT-40 cells. Thus, the kinase, SH2, and PH domains of BTK are all important and apparently indispensable for its function as a negative regulator of Fas-mediated apoptosis. To further characterize the anti-apoptotic function of BTK, we introduced by electroporation full-length wild-type BTK into BTK-deficient cells. Introduction of wild-type BTK protein rendered BTK-deficient DT-40 cells resistant to the apoptotic effects of Fas ligation, suggesting direct protein–protein interactions between BTK and members of the Fas signal transduction pathway as a possible mechanism for the anti-apoptotic function of BTK.

The downstream pro-apoptotic events initiated by the ligation of Fas or TNF receptor-1 are beginning to be illuminated [3, 5–8, 71–78]. Both Fas and TNF receptor-1 contain a homologous intracellular "death domain," which plays a pivotal role in ligand-dependent assembly of a pro-apoptotic DISC [79]. The death domains of p55 TNF receptor-1 and Fas/CD95 serve as docking sites that mediate ligand-dependent recruitment of and heteroassociation with other death domain-containing multivalent adapter proteins: FADD and RIP in the case of CD95; and TRADD and RIP in the case of TNF receptor-1. FADD is the point of convergence between the Fas/CD95- and TNF receptor-1-linked apoptotic signal transduction pathways. Whereas Fas/CD95 directly recruits FADD, TNF receptor-1 binds TRADD, which then acts as an adapter protein to recruit FADD. The formation of CD95–FADD or TNF receptor-1–TRADD–FADD complexes following ligand binding is important for the induction of apoptosis. The assembly of a pro-apoptotic DISC is completed by the recruitment and concomitant activation of the cytosolic caspase FLICE, a member of the ICE protease family [71–78]. Recently, a number of proteins have been identified as inhibitors of Fas- as well as TNFR-1-induced apoptosis. These proteins interact directly with FADD or FLICE, thereby interfering with DISC assembly and function. Notably, the death domain of Fas contains a conserved YXXL motif similar to the immunoreceptor tyrosine-based activation motif (ITAM) sequences as a potential binding site for SH2 containing proteins, and Fas has been shown recently to associate with Fyn and Lck kinases as pro-apoptotic regulators that are required for induction of Fas-mediated apoptosis [80].

We recently investigated if BTK is capable of a physical association with Fas and other members of DISC by examining the Fas, FLICE, FADD, and TRADD immune complexes from the Nonidet P-40 lysates of untreated DT-40 cells for the presence of BTK. BTK was detected by western blot analysis in Fas (but not the other) immune complexes by anti-BTK immunoblotting. Similarly, Fas was detected by anti-Fas immunoblotting in BTK immune complexes from wild-type DT-40 cells as well as BTK-deficient DT-40 cells reconstituted with wild-type human *btk* gene. The constitutive association of BTK with Fas protein was also found in the human pre-B leukemia cell line NALM-6. Taken together, these results demonstrated that BTK is capable of association with Fas protein, and this association does not require prior engagement of the Fas receptor. Fas is associated with FADD in BTK-deficient DT-40 cells, as evidenced by detection of Fas in FADD immune complexes and this physical interaction was enhanced markedly after Fas ligation. In Fas-activated BTK-deficient DT-40 cells, Fas-associated FADD molecules could be detected by anti-FADD immunoblotting. In contrast to BTK-deficient DT-40 cells, very little Fas-FADD association was found in untreated or anti-Fas-treated wild-type DT-40 cells or BTK-deficient DT-40 cells reconstituted with wild-type human BTK. Thus, BTK associates with Fas and prevents its interaction with FADD, which is essential for the recruitment and activation of FLICE by Fas during the apoptotic signal. While these results do not exclude the possibility that BTK may alter the fate of the apoptotic signal triggered by Fas ligation by multiple mechanisms including modulation of the function of positive or negative regulators of apoptotic signal transduction, they do provide at least one plausible explanation for the observed anti-apoptotic function of BTK.

Although the crystal structure of full-length BTK has not been reported, the recently published structures of the PH

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domain and BTK motif [81] provide useful information applicable to the binding capability of BTK and its PH domain. FADD has been reported to interact with the cytoplasmic domain of Fas, which is largely composed of a death domain consisting of six antiparallel α-helices assembled from residues 230–314 [80]. The YXXL sequence of the Fas death domain has been speculated to resemble ITAMs and be recognized by a SH2 domain of a PTK upon tyrosine phosphorylation or by other mechanisms [52, 82, 83]. An analysis of the conformation of this YXXL sequence shows that it is located in the middle of an α-helix and unless a substantial conformational change of that α-helix would occur to make the tyrosine residue more accessible, it may be too rigid for interaction with a PTK. Thus, the structural geometry of the YXXL sequence would likely prevent Fas and BTK from adopting a binding mode such as that of CD3-ε ITAM/ZAP-70, as was suggested [82, 83]. The inability of the BTK SH2 domain to pull down Fas from whole cell lysates further supports this notion. How then does BTK associate with Fas? BTK and Fas may associate via complementary electrostatic attractions and hydrogen bond interactions, which could involve the previously reported charged residues on the surfaces of the α-helices of the Fas death domain. This association could be mediated by a third protein that forms an interface between Fas and BTK. The importance of the SH2 and kinase domains of BTK for its anti-apoptotic function prompts the hypothesis that a tyrosine phosphorylated substrate of BTK may provide such an interface. Further studies will be required to elucidate the exact structural basis for the BTK–Fas interactions.

The ability of BTK to inhibit the pro-apoptotic effects of Fas ligation prompts the hypothesis that apoptosis of developing B-cell precursors during normal human B-cell ontogeny may be reciprocally regulated by Fas and BTK. Inappropriate apoptosis may underlie the pathogenesis as well as drug resistance of human leukemias and lymphomas, which makes control of apoptosis an important potential target for therapeutic intervention. The fate of leukemia/lymphoma cells exposed to chemotherapeutic agents, such as vincristine and daunorubicin, may reside in the balance between the opposing pro-apoptotic effects of caspases activated by DISC and an upstream negative regulatory mechanism involving BTK and/or its substrates. Therefore, inhibitors of BTK are likely to enhance the drug sensitivity of B-lineage leukemia/lymphoma cells.

CONCLUSION AND FUTURE PERSPECTIVE

BTK is the first tyrosine kinase to be identified as a dual function regulator of apoptosis in B-cells, which plays a pivotal role in the initiation of the radiation-induced apoptotic death process but inhibits Fas-activated apoptosis. New pharmacologic agents with BTK-modulatory activity could be useful as radiosensitizing or chemosensitizing agents with clinical potential in the treatment of B-cell malignancies (in particular acute lymphoblastic leukemia, the most common form of childhood cancer) and as B-cell reconstituting agents in humoral immunodeficiencies with decreased numbers or absence of B-cells. As new and more effective forms of gene delivery are developed (e.g monoclonal antibodies, scFv), wild-type *btk* genes could be used as part of radiosensitizing gene therapy programs, whereas kinase-dead "dominant negative" *btk* genes could be used as part of chemosensitizing gene therapy programs. Initial studies on *btk* transcription have demonstrated that expression of the *btk* gene is regulated by the combined action of Sp1- and PU.1-family transcription factors [84, 85]. Transcriptional regulatory elements have been identified within the first and tenth introns of the *btk* gene [86]. Recent studies indicate that regulation of *btk* gene expression is rather complex and likely involves multiple transcription factors [86]. New agents affecting the activity of these transcription factors could also be useful as modulators of apoptotic signals in treatment programs. The feasibility of regulating *btk* gene expression in human hematopoietic cells has already been demonstrated by the ability of retinoic acid to increase *btk* expression in myeloid cells and by the ability of phorbol ester as well as TGF-β1 to decrease *btk* expression in B-cells [87].

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