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Bruton tyrosine kinase is commonly overexpressed in mantle cell lymphoma and its attenuation by Ibrutinib induces apoptosis

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ABSTRACT

Mantle cell lymphoma (MCL) is an aggressive B-cell malignancy that characteristically shows overexpression of cyclin-D1 due to an alteration in the t(11;14)(q13;q32) chromosomal region. Although there are some promising treatment modalities, great majority of patients with this disease remain incurable. The B-cell antigen receptor (BCR) signaling plays a crucial role in B-cell biology and lymphomagenesis. Bruton tyrosine kinase (BTK) has been identified as a key component of the BCR signaling pathway. Evidence suggests that the blockade of BTK activity by potent pharmacologic inhibitors attenuates BCR signaling and induces cell death. Notably, the expression levels and the role of BTK in MCL survival are still elusive. Here, we demonstrated a moderate to strong BTK expression in all MCL cases ($n = 19$) compared to benign lymphoid tissues. Treatment of MCL cell lines (Mino or Jeko-1) with a potent BTK pharmacologic inhibitor, Ibrutinib, decreased phospho-BTK-Tyr^{223} expression. Consistent with this observation, Ibrutinib inhibited the viability of both Mino and JeKo-1 cells in concentration- and time-dependent manners. Ibrutinib also induced a concentration-dependent apoptosis in both cell lines. Consistently, Ibrutinib treatment decreased the levels of anti-apoptotic Bcl-2, Bcl-xL, and Mcl-1 protein. These findings suggest that BTK signaling plays a critical role in MCL cell survival, and the targeting of BTK could represent a promising therapeutic modality for aggressive lymphoma

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1. Introduction

Mantle cell lymphoma (MCL) is characterized by the translocation t(11;14)(q13;q32), which results in the aberrant expression of the cell cycle protein, cyclin-D1 [1–3]. Alteration in DNA damage response genes and activation of cell survival pathways are also implicated in MCL development [4]. MCL accounts for approximately 2–10% of the non-Hodgkin lymphoma cases, with an annual incidence of 0.51–0.55 per 100,000 people [5]. A great majority of MCL patients have stage III/IV disease and present with generalized non-bulky lymphadenopathy, blood and bone marrow involvement, splenomegaly, and extranodal involvement [1,6]. Great majority of patients exhibit an aggressive disease with a median survival 3–5 years [5,7]. These observations strongly suggest the need to develop effective, yet more selective, therapeutic strategies to cure MCL [3,8].

The B-cell antigen receptor (BCR) signaling is crucial to cell survival during B-cell development and regulates multiple biological processes, including cell proliferation, differentiation, apoptosis, and migration [9–11]. In addition, BCR signaling is implicated in the pathogenesis of B-cell malignancies, including MCL [9,12]. Bruton's tyrosine kinase (BTK) is a cytoplasmic protein and has been identified as a key component of the BCR signaling pathway [13]. Signals mediated by BTK were demonstrated to activate cell survival pathways such as transcription factor nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) [9,14]. In addition, the loss-of-function of BTK in humans was linked to X-linked agammaglobulinemia (XLA) syndrome [13]. XLA is an inherited disorder that is characterized by a severe decrease in immunoglobulin production and virtual absence of mature B-cells [14,15]. Since XLA is largely restricted to B-cell lymphocytes, BTK is considered an attractive target for the selective inhibition of B-cell growth [11].

Ibrutinib (PCI-32765) is an orally bioavailable irreversible, potent and highly selective small molecule inhibitor of BTK [15,16]. The results of phase II clinical trail have suggested that Ibrutinib is

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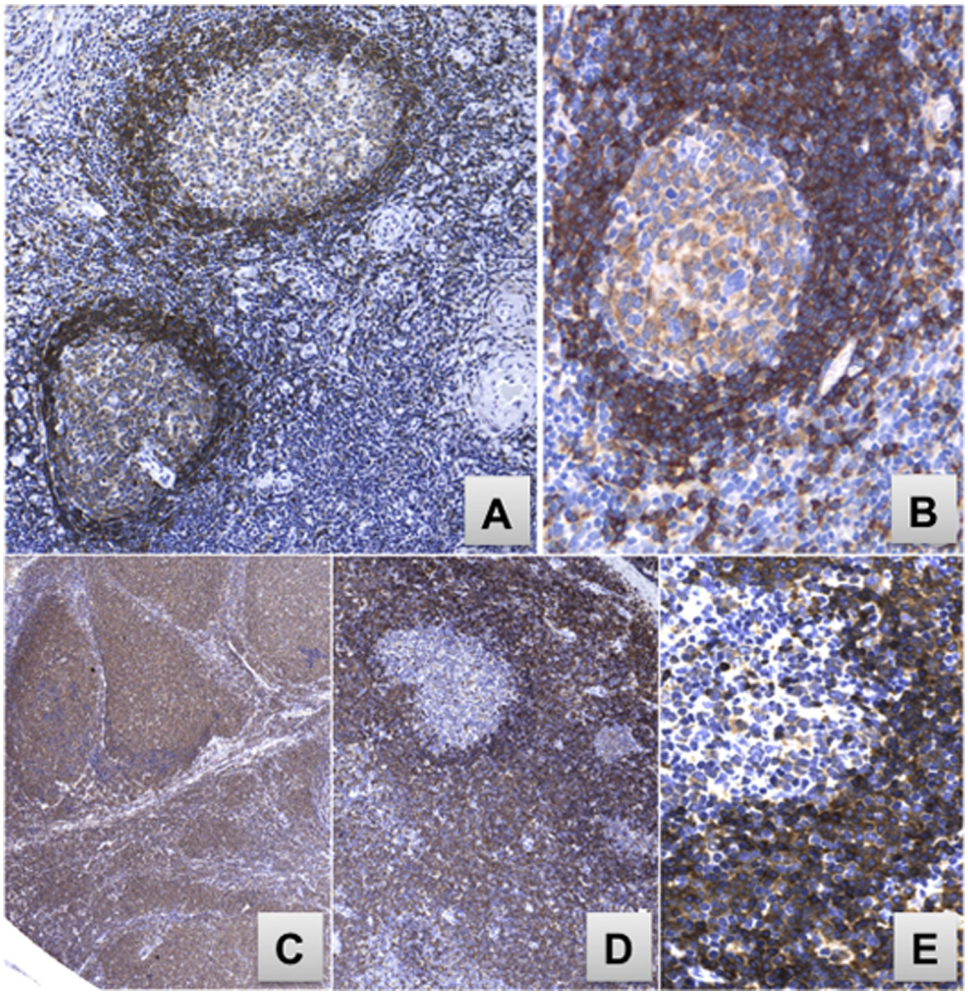

Fig. 1. Immunohistochemical staining of BTK protein in benign lymphoid tissue and mantle cell lymphoma. (A) and (B) BTK expression in benign lymphoid tissue of tonsil with follicular hyperplasia is shown (magnifications A: 100× and B: 400×). Stronger expression of BTK is noticeable in the benign mantle zone cells while a weaker expression noted in the germinal center cells. (C)–(E) Two cases of mantle cell lymphomas are shown. (C) Mantle cell lymphoma primarily with a nodular pattern is illustrating a diffuse expression of BTK by the mantle cell lymphoma cells (magnification: 100×). (D) and (E) Mantle cell lymphoma cells surrounding a residual benign germinal center reveals a strong diffuse cytoplasmic and membranous staining of BTK (D: 100× and E:400×). Micrographs are the representation of multiple images.

likely very effective and well tolerated in relapsed and refractory MCL and the efficacy of this drug is being evaluated in Phase 3 trials [7,10]. Despite these findings, however, the expression of BTK in normal and neoplastic mantle cells as well as the effects of BTK in MCL cell survival has not been systematically assessed.

In the present study, we demonstrated that BTK is commonly overexpressed in MCL compared to benign lymph nodes, and the inhibition of BTK by Ibrutinib attenuated MCL cell growth and survival by a mechanism that involved in the inhibition of anti-apoptotic proteins. These findings suggest that BTK contributes to MCL survival and provide a rationale for the development of novel therapeutic approaches that include BTK inhibitory agents.

2. Materials and methods

2.1. Cell lines and reagents

Mino and JeKo-1 cell lines were grown in RPMI 1640 as described previously [17]. Ibrutinib was obtained from Selleckchem (Houston, TX, USA). Bcl-2 (C-2) PE, Bcl-xL (H-5) FITC, Mcl-1 (H-260), and goat anti-rabbit IgG-PE antibodies were purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Total and phospho-Tyr^{223}BTK antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Propidium iodide (PI) was purchased from Sigma-Aldrich and 7-amino-actinomycin (7-AAD Viability Dye) from Beckman Coulter (Immunotech, Inc.).

2.2. Immunohistochemistry

Archival formalin-fixed, paraffin-embedded tissue blocks of B-cell lymphoma and benign lymphoid tissue were subjected to immunohistochemistry with BTK on 19 MCL cases along with 10 cases of benign lymphoid tissues. Immunostaining was performed with an automated immunostainer (Leica microsystem). The staining protocol used a rabbit monoclonal antibody against BTK as described by the manufacturer (Cell Signaling Technology). The sections were microscopically evaluated for the intensity of reactivity. The results were recorded in semi quantitative fashion categorized as negative (0), weakly positive (1) or strongly positive (2) cytoplasmic expression in tumor cells based on intensity of the immunostaining.

2.3. Flow cytometry

For the analysis of phospho-BTK-Tyr^{223}, Bcl-2, Bcl-xL, and Mcl-1, cells were processed using intracellular staining kit (Fix & Perm kit, Invitrogen). Cells were labeled with phospho-BTK-Tyr^{223} antibody (1:10 dilution) or with Mcl-1 (1:2 dilutions) for 20 min and washed with PBS, incubated with phycoerythrin-conjugated secondary antibody (goat anti-rabbit IgG-PE) for 30 min at 4 °C, and then washed with PBS before the analysis. Processed-cells were directly stained with Bcl-2-PE or Bcl-xL-FITC conjugated antibody and washed with PBS to remove unbound antibody. Analyses of phospho-BTK-Tyr^{223} at 24 h post treatment and of Bcl-2-PE, Bcl-xL-FITC, or Mcl-1 at 72 h post treatment were performed using Cytomic's FC500 Flow Cytometer (Beckman Coulter).

2.4. Cell viability assay

The viability of Mino and JeKo-1 cells was determined by MTS tetrazolium compound (PromegaCellTiter 96® AQ$_{ueous}$ One Solution Cell Proliferation Assay;

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