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BAFF activates Erk1/2 promoting cell proliferation and survival by Ca^{2+}-CaMKII-dependent inhibition of PP2A in normal and neoplastic B-lymphoid cells

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ABSTRACT

B-cell activating factor (BAFF) is involved in not only the physiology of normal B cells, but also the pathophysiology of aggressive B cells related to malignant and autoimmune diseases. However, how excessive BAFF promotes aggressive B-cell proliferation and survival is not well understood. Here we show that excessive human soluble BAFF (hsBAFF) enhanced cell proliferation and survival in normal and B-lymphoid (Raji) cells, which was associated with suppression of PP2A, resulting in activation of Erk1/2. This is supported by the findings that pretreatment with U0126 or PD98059, expression of dominant negative MKK1, or overexpression of PP2A prevented hsBAFF-induced activation of Erk1/2 and cell proliferation/viability in the cells. It appears that hsBAFF-mediated PP2A-Erk1/2 pathway and B-cell proliferation/viability was Ca^{2+}-dependent, as pretreatment with BAPTA/AM, EGTA or 2-APB significantly attenuated these events. Furthermore, we found that inhibiting CaMKII with KN93 or silencing CaMKII also attenuated hsBAFF-mediated PP2A-Erk1/2 signaling and B-cell proliferation/viability. The results indicate that BAFF activates Erk1/2, in part through Ca^{2+}-CaMKII-dependent inhibition of PP2A, increasing cell proliferation/viability in normal and neoplastic B-lymphoid cells. Our data suggest that inhibitors of CaMKII and Erk1/2, activator of PP2A or manipulation of intracellular Ca^{2+} may be exploited for prevention of excessive BAFF-induced aggressive B-cell malignancies and autoimmune diseases.

1. Introduction

The TNF superfamily plays a crucial role in the regulation of immune response by inducing apoptosis and/or proliferation in lymphocytes [1]. B-cell activating factor of the TNF family (BAFF), also termed BLyS, TALL-1, THANK, and zTNF4, a type II membrane protein that exists in both membrane-bound and soluble forms, is a ligand for three TNF-receptor-family members: BAFF-R (BR3), BCMA, and TACI [2–7]. BAFF has exhibited a strong co-stimulatory function in development, maturation and homeostasis of normal B lymphocytes, as well as in cell proliferation and survival of neoplastic B-lymphoid cells [8–11]. Systemic administration of soluble BAFF results in B-cell expansion and elevated levels of immunoglobulins [7]. More importantly, high levels of BAFF in the serum of mice that express both endogenous and transgenic BAFF especially extend B lymphocyte survival beyond physiological limits, and drive continued production of plasma cells producing pathogenic autoantibodies, which contribute to systemic lupus

Abbreviations: 2-APB, 2-aminoethoxydiphenyl borate; BAFF, B-cell activating factor of the TNF family; BAPTA/AM, 1,2-bis(o-aminophenoxy) ethane-N,N,N′,N′-tetra-acetic acid tetra(acetoxymethyl) ester; BLyS, B lymphocyte stimulator; BCMA, B cell maturation antigen; CaM, calmodulin; CaMKII, calcium/calmodulin-dependent protein kinase II; CRAC, Ca^{2+}-release activated Ca^{2+}; EGTA, ethylene glycol tetra-acetic acid; ER, endoplasmic reticula; Erk1/2, extracellular signal-related kinase 1/2; FBS, fetal bovine serum; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; PBS, phosphate buffered saline; PP2A, protein phosphatases 2A; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; TACI, transmembrane activator and cyclophilin ligand interactor; TALL-1, TNF and apoptosis ligand-related leukocyte-expressed ligand1; THANK, TNF homologue that activates apoptosis, nuclear factor κB, and c-Jun NH2-terminal kinase.

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erythematosus (SLE) pathogenesis [12–14]. The B cells with prolonged lifespan are considered culprits in developing lupus-like autoimmune diseases [9,14–16]. In humans, increased serum BAFF levels are found in a number of different autoimmune diseases, such as SLE, rheumatoid arthritis (RA), and Sjögren's syndrome (SS) [1,14,17]. These results indicate that increased expression of BAFF is a possible etiological factor of aggressive or neoplastic B-cell disorders and autoimmune diseases. However, how excessive BAFF promotes aggressive B-cell proliferation and survival is not well understood.

Extensive studies have shown that BAFF regulates expression of several Bcl-2 family members, including Bcl-x_L, Mcl-1, A1/Bfl-1, Bcl-2, and Bim, via survival-promoting kinase systems such as Pim 1/2 or extracellular signal-related kinases 1/2 (Erk1/2) [11]. Protein phosphatase 2A (PP2A), a ubiquitous and highly conserved serine/threonine (Ser/Thr) protein phosphatase, plays an essential role in multiple cellular processes, including cell proliferation/growth and death, cell mobility, cytoskeleton dynamics, as well as numerous signaling pathways [18–20]. PP2A negatively regulates Erk1/2 pathway through dephosphorylating and inactivating both mitogen-activated protein kinase kinases 1/2 (MEK1/2) and Erk1/2 proteins [18]. Dysregulation of PP2A activity has been implicated in several diseases. For example, in leukemic cells, inhibition of PP2A activity increases proliferation and impairs cellular differentiation [21]. Increased PP2A activity exists in T cells from patients with SLE [22]. PP2A overexpression promotes DNA hypomethylation through suppressing MEK/Erk/DNA methyltransferase 1(DNMT1) pathway in normal and SLE T-cells [20]. However, decreased PP2A activity in the brain of Alzheimer's patients promotes hyperphosphorylation of tau protein leading to the development of neurofibrillary tangles [23]. Although an important role for PP2A has been established in a number of disease processes, including SLE [20,24,25], its effects on BAFF-induced proliferation and survival of aggressive B cells are unclear.

Calcium ion (Ca^{2+}) is a ubiquitous intracellular signal responsible for numerous cellular events, such as proliferation/growth, differentiation, and survival in various immune

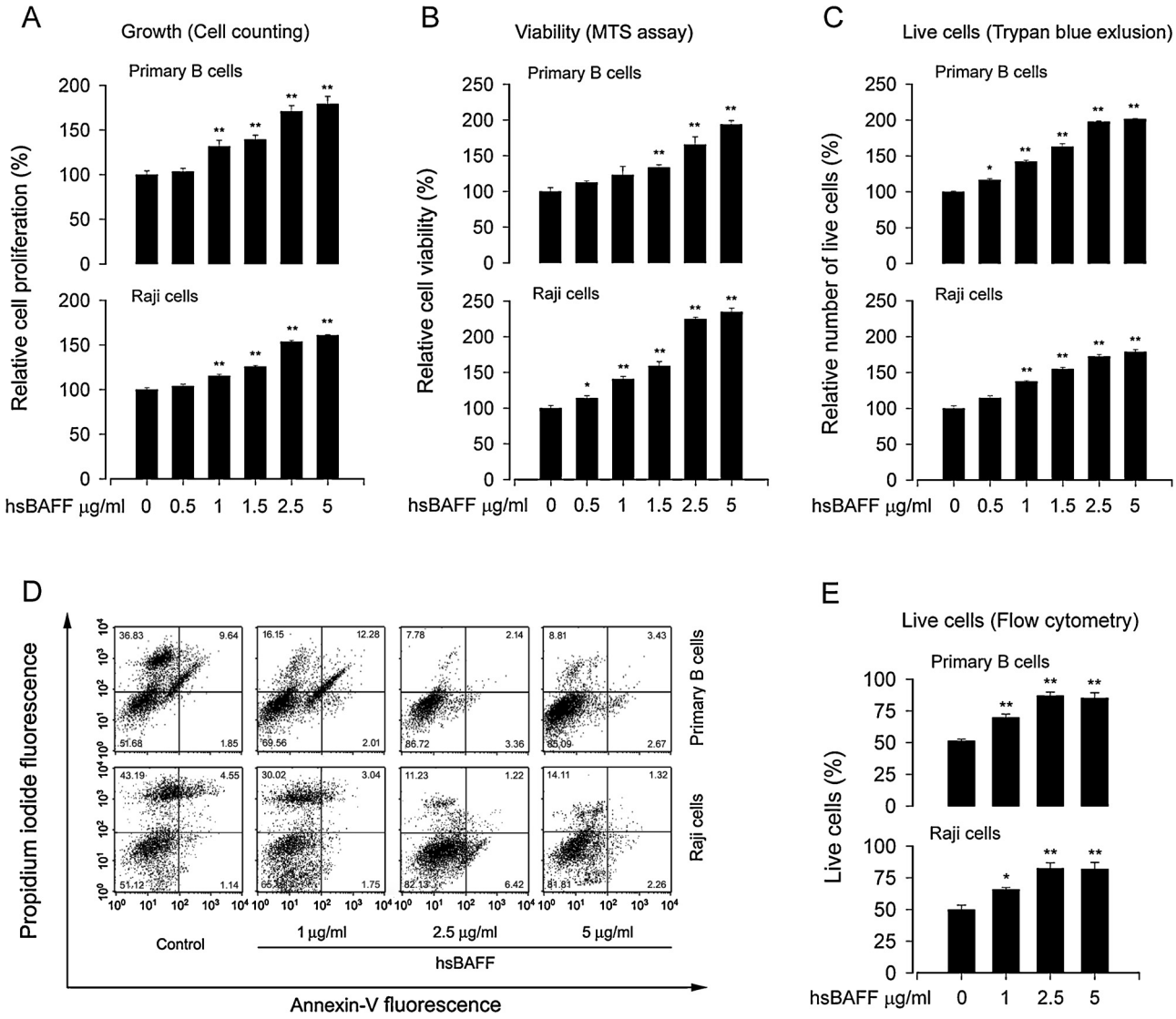

Fig. 1. hsBAFF promotes normal and Raji B-cell proliferation and viability. Raji cells and purified mouse splenic B lymphocytes were treated with 0–5 μg/mL hsBAFF for 48 h. (A) Cell proliferation was evaluated by cell counting. (B) Cell viability was monitored by measuring OD at 490 nm using MTS reagents. (C) Live cells were detected by counting viable cells using trypan blue exclusion. (D) The ratios of death cells, live cells, necrotic and apoptotic cells were calculated by FACS using annexin-V-FITC and propidium iodide staining. Results from one representative experiment are shown. (E) Quantitative analysis of live cells by FACS assay. Results are presented as mean ± SE (n = 3–6). $^{*}P < 0.05$, $^{**}P < 0.01$, difference *vs* control group.