Biochemical and Biophysical Research Communications 425 (2012) 854–858

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

BAFF induces spleen CD4$^+$ T cell proliferation by down-regulating phosphorylation of FOXO3A and activates cyclin D2 and D3 expression

Fang Ji [a], Rongjing Chen [a], Baojun Liu [b], Xiaoping Zhang [c], Junli Han [d], Haining Wang [d], Gang Shen [a,*], Jiang Tao [d,*]

[a] Department of Orthodontics, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Stomatology, Shanghai, China
[b] Laboratory of Lung, Inflammation and Cancers, Huashan Hospital, Fudan University, Shanghai, China
[c] Department of Nuclear Medicine, Shanghai 10th People's Hospital, Tongji University School of Medicine, Shanghai 200072, China
[d] Department of General Dentistry, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Stomatology, Shanghai, China

ARTICLE INFO

Article history:
Received 23 July 2012
Available online 7 August 2012

Keywords:
CD4$^+$ T cells
Cyclin D2
Cyclin D3
Spleen
Phosphorylated FOXO3A

ABSTRACT

The TNF ligand family member "B cell-activating factor belonging to the TNF family" (BAFF, also called BLyS, TALL-1, zTNF-4, and THANK) is an important survival factor for B and T cells. In this study, we show that BAFF is able to induce CD4$^+$ spleen T cell proliferation when co-stimulated with anti-CD3. Expression of phosphorylated FOXO3A was notably down-regulated and cyclins D2 and D3 were up-regulated and higher in the CD4$^+$ T cells when treated with BAFF and anti-CD3, as assessed by Western blotting. Furthermore, after FOXO3A was knocked down, expression of cyclin D1 was unchanged, compared with control group levels, but the expression of cyclins D2 and D3 increased, compared with the control group. In conclusion, our results suggest that BAFF induced CD4$^+$ spleen T cell proliferation by down-regulating the phosphorylation of FOXO3A and then activating cyclin D2 and D3 expression, leading to CD4$^+$ T cell proliferation.

1. Introduction

BAFF is a ligand for three TNF receptor family members, BAFF-R (BR3), TACI, and BCMA, and is expressed on macrophages/monocytes, dendritic cells, and T lymphocytes [1–5]. BAFF in trimeric form plays an important role as a co-stimulator of T cell function, such as in T-cell activation, proliferation, and cytokine production *in vitro*, and especially in CD4$^+$ and CD8$^+$ T lymphocytes in mice spleen [5,6]. The activity of BAFF on T lymphocyte responses is further strengthened by the observations that hsBAFF results in a significantly increased population of splenic CD4$^+$ T lymphocytes [6–8]. However, the exact mechanism by which BAFF induces CD4$^+$ T cell proliferation remains unclear.

The forkhead transcription factor (FOXO) subfamily consists of FOXO3A (FKHRL1), FOXO1A (FKHR), and FOXO4 (AFX), all downstream effectors of the PTEN/PI3K/AKT pathway [9]. These FOXOs have been found to participate in a growing number of physiological processes, including cell proliferation, apoptosis, stress resistance, differentiation, and metabolism [10].

Phosphorylation of FOXO3A prevents its transcriptional repression of targets that promote cell proliferation, such as D-type cyclins. Additionally, FOXO3A has been shown to function at the G2-M checkpoint in the cell cycle and to trigger repair of DNA damage by preventing D-type cyclin activity [11,12]. Three D-type cyclins (D1, D2, D3), which are involved with mammalian G1 cyclins and their associated kinases, are regulators of the cell division cycle that integrate information flow from outside the cell to drive G1-phase progression and initiate DNA replication in response to mitogenic signals [13,14].

Cyclin D2 is an essential mediator of BCR-induced proliferation of mature B lymphocytes and may be an indicator of an alternate pathway for the development of IgM B cells [15]. Moreover, IL-12 up-regulates the expression of the cell cycle-related molecule cyclin D3 to provide CD4$^+$ T cell proliferation [16]. However, there is no report of the effects of FOXO3A on D-type cyclins in CD4$^+$ spleen T cells.

In this study, we first demonstrated that BAFF was one of the important cytokines induced in CD4$^+$ spleen T cell proliferation by the FOXO3A signaling protein. Additionally, we found that the mechanism involved BAFF down-regulating phosphorylation of FOXO3A and then activating cyclin D3 expression, leading to CD4$^+$ T cell proliferation.

* Corresponding authors. Addresses: Department of Orthodontics, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Stomatology, No. 639 Zhi Zao Ju Road, Shanghai 200011, China. Fax: +86 21 23271699 5206 (G. Shen). Department of General Dentistry, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Stomatology, No. 639 Zhi Zao Ju Road, Shanghai 200011, China. Fax: +86 21 23271699x5202 (J. Tao).
E-mail address: taojiang2012@yahoo.cn (J. Tao).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.08.003

2. Materials and methods

2.1. Preparation of $CD4^+$ T lymphocytes

ICR mice, each weighing 20–25 g, were obtained from Shanghai Laboratory Animal Center, Chinese Academy Sciences, Shanghai, China. The animals were sacrificed by cervical dislocation and spleens were collected under sterile conditions. Spleen cells were collected by grinding and filtering through sterilized nylon membranes and were washed in Hank's solution (pH 7.2) and then isolated using a discontinuous polyvinylpyrrolidone silica (Percoll) gradient (Pharmacia, Piscataway, NJ, USA). T lymphocytes of >95% purity were isolated from splenic cell suspensions using the MagCellect* Mouse $CD3^+$ T cell isolation kit according to the manufacturer's protocol. $CD4^+$ T lymphocytes of >95% purity were isolated from splenic cell suspensions using BD IMag Anti-Mouse CD4 Particles (magnetic) according to the manufacturer's protocol. Cells were resuspended in RPMI 1640 medium containing 10% FBS and 100 U/mL penicillin/streptomycin for further experiments.

2.2. Cell proliferation assay

Purified $CD4^+$ T lymphocytes were diluted to 4×10^5 cells/mL and seeded at 0.1 mL/well in 96-well flat-bottomed plates; they were activated with 1 μg/mL anti-CD3 immobilized on plastic surfaces. The next day, cells were randomly divided into a normal control group and two treatment groups. Recombinant human BAFF was from Peprotech Inc. (Rocky Hill, NJ, USA; 1 or 2.5 μg/mL); cells were treated in triplicate in each group. Cultures were maintained for 48 h at 37 °C with 5% CO_2/humidified air. Then, 0.5 μCi of [3H]-thymidine was added to each well, and the cells were further incubated for 4 h. The final incorporation of [3H]-thymidine into cells was measured in a liquid scintillation counter (LS-6500, Beckman Instruments, Inc., Pullerton, CA, USA). The cell viability in all treatment groups was confirmed to be comparable to that in the control group by trypan blue exclusion assay.

2.3. siRNA-mediated Foxo3a gene knock-out

To silence *Foxo3a* gene expression, we performed transfection of a siRNA duplex using GeneTrans II (MoBiTec). According to previous studies [17–19], a Foxo3a small interfering RNA (siRNA) was synthesized by Invitrogen Corp., Shanghai, China, using the following sequences: 5′-GGA UGA CGU CCA GGA UGA UTT-3′ (sense) and 5′-AUC AUC CUG GAC GUC AUC CTT-3′ (anti-sense). A nonspecific siRNA (NC siRNA) was also synthesized by Invitrogen Corp.; the sequences were 5′-UUC UCC GAA CGU GUC ACG-3′ (sense) and 5′-ACG UGA CAC GUU CGG AGA ATT-3′ (anti-sense). The Foxo3a siRNA was transfected into $CD4^+$ T cells using the transfection reagent INTERFERin (Dakewe Corp., France), according to the manufacturer's protocol. The transfection efficiency was examined by Western blotting, as described below, at 12 and 24 h post-transfection. Furthermore, to detect the relationship between BAFF and FOXO3A, first, $CD4^+$ T cells were transferred into nucleofection buffer. Next, BAFF (2.5 μg/mL) and 1 μg/mL anti-CD3 was added 2 h after transfection. Then, at 24 h after transfection, the proliferation of $CD4^+$ T cells and expression of D-type cyclins were assessed using MTT and Western blotting, respectively.

2.4. Immunoblot analysis

After harvesting, cells were washed in phosphate-buffered saline (PBS) and lysed in 1 × sodium dodecylsulfate (SDS) loading buffer. Lysates were then clarified by centrifugation (15,000*g*, 10 min, 4 °C) and collected. Protein concentrations were determined using the Bradford assay (Bio-Rad, Hercules, CA, USA). Equivalent amounts of protein (20–50 μg) were separated by SDS–PAGE; briefly, proteins were resolved using 12% SDS–polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). Membranes were incubated with PBS containing 0.05% Tween 20 and 5% nonfat dry milk to block nonspecific binding and were incubated with appropriate rabbit polyclonal antibodies against FOXO3A (SC-9812), p-FOXO3A (SC-12897), cyclin D1 (SC-450), cyclin D2 (SC-593), cyclin D3 (SC-182), and β-actin (SC-81178) were purchased from Santa Cruz Biotechnology. Then, blots were washed and incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin (Ig)G, washed again, and immunoreactivity was then detected by chemiluminescence methods. For all immunoblots, β-actin immunoreactivity was used as a loading control. All antibodies were used at a dilution of 1:2000. Western blot analyses were performed in three independent experiments. Blots were then exposed to radiographic film to visualize immunoreactive signals. All antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), and signals were quantified using Multi Gauge Image Analysis software (FujiFilm, Japan).

2.5. Statistical analyses

Statistical analyses were performed using the SPSS software. Differences between groups were evaluated by one-way analysis of variance (ANOVA) with Tukey's test. *P* values of less than 0.05 were considered to indicate statistical significance.

3. Results

3.1. BAFF induced $CD4^+$ T cell proliferation

As shown in Fig. 1, BAFF significantly enhanced the proliferation of purified mouse splenic $CD4^+$ T cells treated with 1 or 2.5 μg/mL BAFF and co-stimulated with an optimum dose anti-CD3 (1 μg/mL) when detected thymidine incorporation ($p < 0.001$). Additionally, the effects of 1–2.5 μg/mL BAFF were concentration-dependent, indicating that BAFF induced $CD4^+$ T cell proliferation *in vitro*.

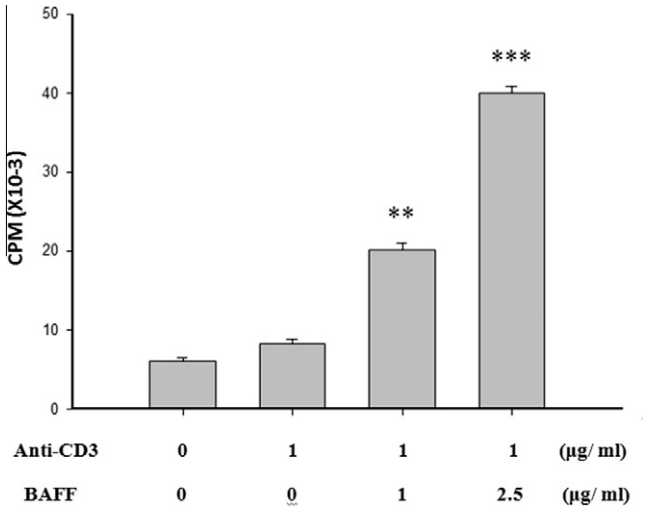

Fig. 1. BAFF induced $CD4^+$ T cell proliferation by detect thymidine incorporation. Columns represent means of triplicates from one representative experiment; error bars indicate means ± standard error (SE). $^{**}p < 0.01$, $^{***}p < 0.001$, versus the control group.

3.2. Expression of FOXO3A and D-type cyclins in CD4$^+$ T cells, assessed using Western blotting

To address the effects of BAFF on the expression of FOXO3A and D-type cyclins in CD4$^+$ T cell, we conducted Western blots to assess changes in these proteins when the purified mouse splenic CD4$^+$ T cells were treated with 2.5 μg/mL BAFF and were co-stimulated with an optimum dose anti-CD3 (1 μg/mL). As shown in Fig. 2A, FOXO3A expression was unchanged, while the level of phosphorylated FOXO3A was notably down-regulated in the CD4$^+$ T cells when treated with BAFF and anti-CD3 ($p < 0.01$). Accordingly, in Fig. 2B, two closely migrating bands, one more strongly immunoreactive than the other, were labeled with the cyclin D1 antiserum. Expression of cyclin D1 was unchanged, when compared with levels in the control group, but the expression of cyclins D2 and D3 was up-regulated and higher than the control group ($p < 0.05$ and $p < 0.01$, respectively). Together, these results indicate that BAFF prevented phosphorylation of FOXO3A and induced expression of cyclins D2 and D3 protein in CD4$^+$ T cells, suggesting the enhanced CD4$^+$ T cell proliferation may be mediated through a FOXO3A and cyclins D2 and D3 signaling pathway.

3.3. siRNA-mediated Foxo3a gene knock-out

To further explore whether FOXO3A played an important role in BAFF-induced CD4$^+$ T cell proliferation, we used siRNA to knock down the expression of FOXO3A. As shown in Fig. 3A, the transfection efficiency of siRNA in CD4$^+$ T cells was more than 70%. The Foxo3a siRNA reduced the level of FOXO3A at 24 h post-transfection. Then, we analyzed BAFF- and anti-CD3-induced CD4$^+$ T cell proliferation after FOXO3A was knocked down. As described in the materials and methods, BAFF (2.5 μg/mL) and anti-CD3 (1 μg/mL) were added to the CD4$^+$ T cell medium 24 h after transfection of the Foxo3a or NC siRNA. Then, 24 h later, proliferation was analyzed using the MTT method. As shown in Fig. 3B, after FOXO3A was down-regulated, proliferation of CD4$^+$ T cell treated with BAFF and anti-CD3 increased significantly, compared with the control group ($p < 0.001$, Fig 3B). We also detected the expression of D-type cyclins in CD4$^+$ T cells treated with BAFF and anti-CD3 after FOXO3A was knocked down. Expression of cyclin D1 was unchanged, compared with control group levels, but the expression of cyclins D2 and D3 was markedly increased, compared with the control group and FOXO3A knocked down group (Fig. 3C).

4. Discussion

In this study, we analyzed the effects of BAFF and anti-CD3 co-stimulation on purified mouse splenic CD4$^+$ T cells by Western blot analyses. We first showed that BAFF induced CD4$^+$ spleen T cell proliferation by down-regulating phosphorylation of FOXO3A and then activating cyclin D2 and D3 expression, leading to CD4$^+$ T cell proliferation. However, this did not appear to involve cyclin D1.

Accumulating experimental evidence supports the notion that BAFF plays important roles in T cell function [5–8]. We showed that BAFF significantly enhanced the proliferation of purified mouse splenic CD4$^+$ T cells in a concentration-dependent manner. These findings were consistent with previous reports [5,7,8] and further suggest that BAFF is an importance immune function regulator not only in B lymphocytes, but also T cells.

We also investigated the mechanism of BAFF-induced CD4$^+$ T cell proliferation. FOXO3A, the main isoform of FOXO proteins in mammals, has been studied in tumorigenesis, muscle atrophy, immune diseases, and stem cell homeostasis. Phosphorylation of FOXO3A is the most important post-translational modification because it essentially regulates the translocation of FOXO3A protein

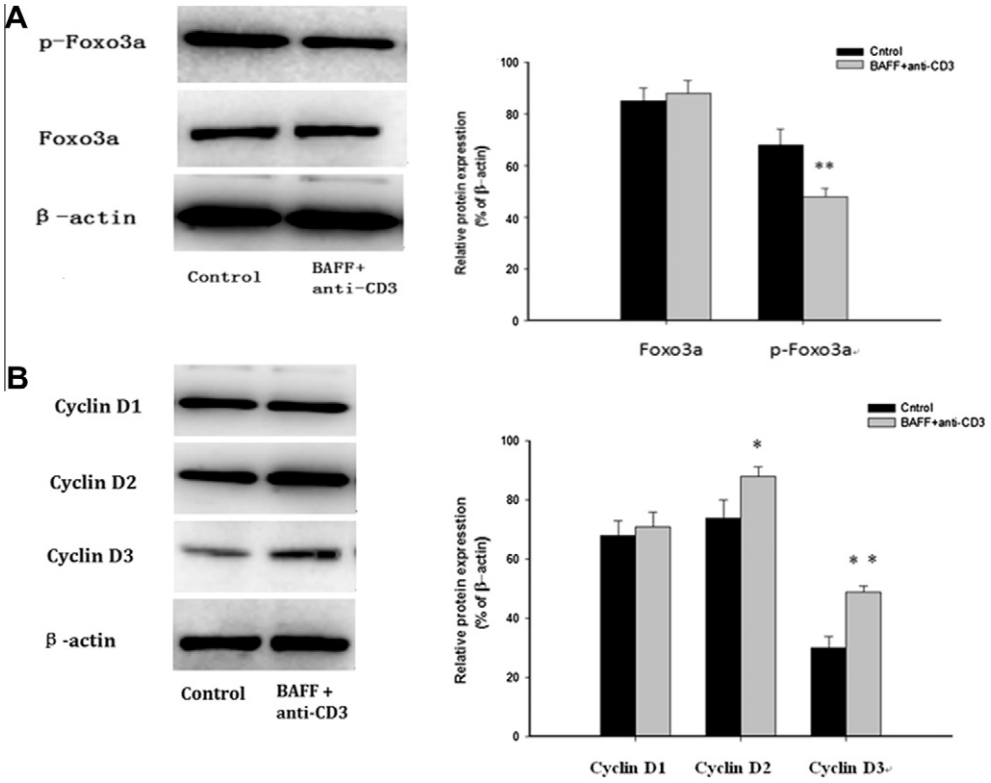

Fig. 2. Expression of FOXO3A and D-type cyclins in CD4$^+$ T cells treated with BAFF and co-stimulated anti-CD3 by Western blot analysis. A. Expression of FOXO3A in CD4$^+$ T cells. B. Expression of D-type cyclins in CD4$^+$ T cells. Columns indicate the means of triplicates in one representative experiment; error bars indicate means ± standard error (SE). $^*p < 0.05$, $^{**}p < 0.01$, versus the control group.

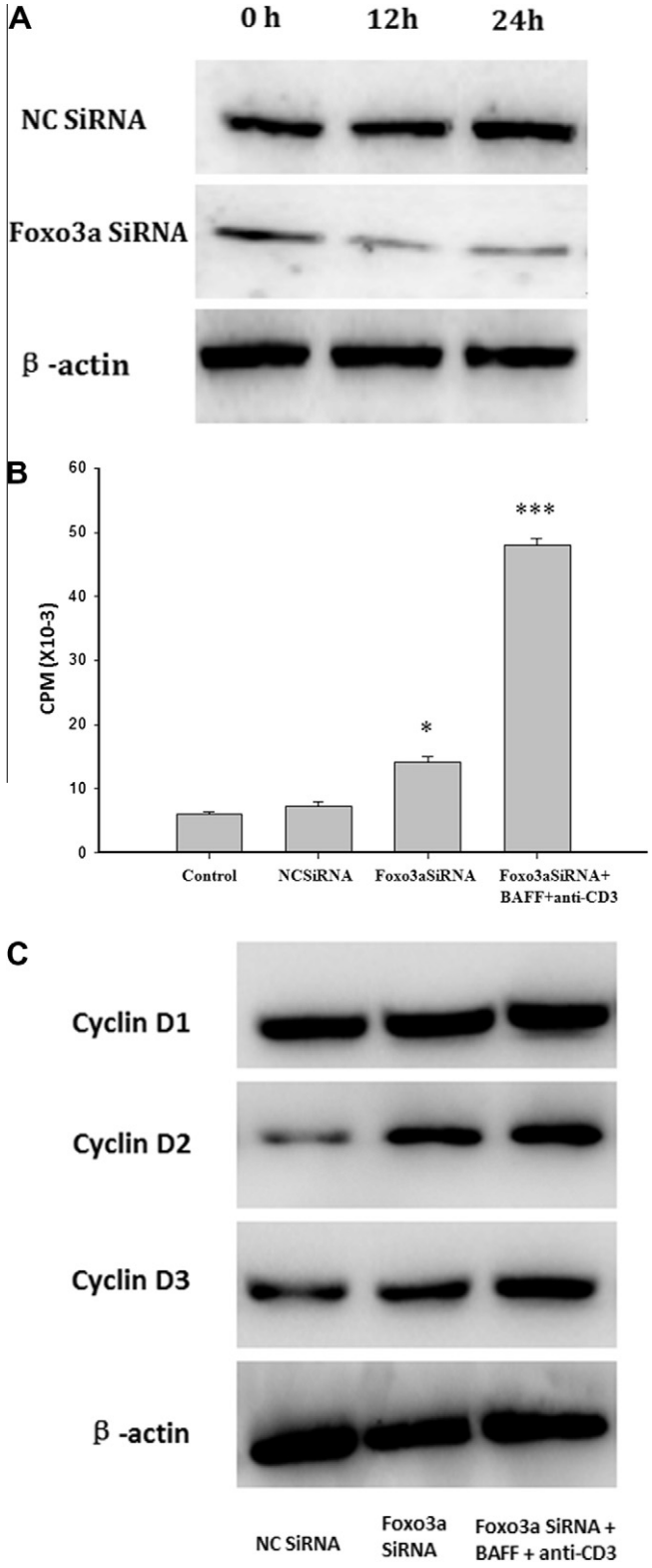

Fig. 3. siRNA to knock down the expression of FOXO3A and D-type cyclins. (A) Transfection efficiency of Foxo3a siRNA in $CD4^+$ T cells. (B) BAFF and anti-CD3-induced $CD4^+$ T cell proliferation after FOXO3A was knocked down. (C) Expression of D-type cyclins in the $CD4^+$ T cell in different treatment. Columns indicate the means of triplicates in one representative experiment; error bars indicate means ± standard error (SE). $^*p < 0.05$, $^{***}p < 0.001$, versus the control group.

between the nucleus and cytoplasm [20,21]. Phosphorylated FOXO3A in B cells was transferred out of the nucleus and then induced cell proliferation via the pre-BCR [22]. In our study, we observed that the expression of phosphorylated FOXO3A was notably up-regulated in $CD4^+$ T cells when treated with BAFF and anti-CD3 (Fig. 2A). Furthermore, the proliferation was significantly decreased, compared with the control group, after FOXO3A was down-regulated by siRNA interference. Thus, these findings suggest that BAFF induced $CD4^+$ spleen T cell proliferation by down-regulating phosphorylation of FOXO3A.

Cell proliferation requires the expression of cyclin D, which is a key component for facilitating the G1 to S phase transition and subsequently increased cell proliferation. There are three members of the cyclin D family (D1, D2, D3), all of which play roles in G1 progression [23]. Thus, we examined the expression of D-type cyclins. Interestingly, the expression of cyclins D2 and D3 was up-regulated and higher than the control group when $CD4^+$ T cells were treated with BAFF and anti- CD3, but cyclin D1 was unchanged (Fig. 2C).

As described in previous reports, different D cyclins are required for tumorigenesis in cyclin-specific tissues (e.g., cyclin D1, breast; cyclin D3, immature T cells). Knockdown of cyclin D3 inhibited proliferation of T cell acute lymphoblastic leukemia (T-ALL) cells [24–26]. Thus, our findings indicate that cyclins D2 and D3 played a major role in BAFF-induced $CD4^+$ spleen T cell proliferation. However, the involvement of cyclin D1 in the exact mechanism needs further study.

Notably, we found that the expression of cyclins D2 and D3 decreased, compared with the control group, after FOXO3A siRNA interference and treated with BAFF and anti-CD3 (Fig. 3C). Recently, FOXO3A was shown to induce withdrawal from the cell cycle, in the G1 phase, and this was the result of increased transcription of the cdk inhibitor p27kip1 and involves down-regulation of cyclins D1 and D2 [11,27,28]. Our findings are largely consistent with this, but further research is needed to explore the role of cyclin D3.

Acknowledgments

This work was supported by Science and Technology Commission of Shanghai (10411964500 and 11411951800), Shanghai Municipal Education Commission (10YZ33).

References

[1] C. Bossen, P. Schneider, BAFF, APRIL and their receptors: structure, function and signaling, Semin. Immunol. 18 (2006) 263–275.
[2] F. Mackay, C.R. Mackay, The role of BAFF in B-cell maturation, T-cell activation and autoimmunity, Trends Immunol. 23 (2002) 113–115.
[3] F. Mackay, P. Schneider, P. Rennert, J. Browning, BAFF AND APRIL: a tutorial on B cell survival, Annu. Rev. Immunol. 21 (2003) 231–264.
[4] P.A. Moore, O. Belvedere, A. Orr, K. Pieri, D.W. LaFleur, P. Feng, D. Soppet, M. Charters, R. Gentz, D. Parmelee, Y. Li, O. Galperina, J. Giri, V. Roschke, B. Nardelli, J. Carrell, S. Sosnovtseva, W. Greenfield, S.M. Ruben, H.S. Olsen, J. Fikes, D.M. Hilbert, BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator, Science 285 (1999) 260–263.
[5] P. Schneider, The role of APRIL and BAFF in lymphocyte activation, Curr. Opin. Immunol. 17 (2005) 282–289.
[6] X.Z. Xia, J. Treanor, G. Senaldi, S.D. Khare, T. Boone, M. Kelley, L.E. Theill, A. Colombero, I. Solovyev, F. Lee, S. McCabe, R. Elliott, K. Miner, N. Hawkins, J. Guo, M. Stolina, G. Yu, J. Wang, J. Delaney, S.Y. Meng, W.J. Boyle, H. Hsu, TACI is a TRAF-interacting receptor for TALL-1, a tumor necrosis factor family member involved in B cell regulation, J. Exp. Med. 192 (2000) 137–143.
[7] X. Shan, L. Chen, M. Cao, L. Xu, S. Zhang, Effects of human soluble BAFF synthesized in *Escherichia coli* on $CD4^+$ and $CD8^+$ T lymphocytes as well as NK cells in mice, Physiol. Res. 55 (2006) 301–307.
[8] L. Wen, S.J. Chen, W. Zhang, H.W. Ma, S.Q. Zhang, and L. Chen, hsBAFF regulates proliferation and response in cultured CD4(+) T lymphocytes by upregulation of intracellular free Ca(2+) homeostasis. *Cytokine* 53 215–22.
[9] H. Tran, A. Brunet, E.C. Griffith, and M.E. Greenberg, The many forks in FOXO's road. *Sci.* STKE 2003 (2003) RE5.
[10] J. Nakae, W.H. Biggs 3rd, T. Kitamura, W.K. Cavenee, C.V. Wright, K.C. Arden, D. Accili, Regulation of insulin action and pancreatic beta-cell function by mutated alleles of the gene encoding forkhead transcription factor Foxo1, Nat. Genet. 32 (2002) 245–253.

[11] M. Schmidt, S. Fernandez de Mattos, A. van der Horst, R. Klompmaker, G.J. Kops, E.W. Lam, B.M. Burgering, R.H. Medema, Cell cycle inhibition by Foxo forkhead transcription factors involves downregulation of cyclin D, Mol. Cell. Biol. 22 (2002) 7842–7852.
[12] H. Tran, A. Brunet, J.M. Grenier, S.R. Datta, A.J. Fornace Jr., P.S. DiStefano, L.W. Chiang, M.E. Greenberg, DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein, Science 296 (2002) 530–534.
[13] T.C. Chiles, Regulation and function of cyclin D2 in B lymphocyte subsets, J. Immunol. 173 (2004) 2901–2907.
[14] M.A. Ciemerych, A.M. Kenney, E. Sicinska, I. Kalaszczynska, R.T. Bronson, D.H. Rowitch, H. Gardner, P. Sicinski, Development of mice expressing a single D-type cyclin, Genes Dev. 16 (2002) 3277–3289.
[15] N. Solvason, W.W. Wu, D. Parry, D. Mahony, E.W. Lam, J. Glassford, G.G. Klaus, P. Sicinski, R. Weinberg, Y.J. Liu, M. Howard, E. Lees, Cyclin D2 is essential for BCR-mediated proliferation and CD5 B cell development, Int. Immunol. 12 (2000) 631–638.
[16] J.M. Grayson, K. Murali-Krishna, J.D. Altman, R. Ahmed, Gene expression in antigen-specific $CD8^+$ T cells during viral infection, J. Immunol. 166 (2001) 795–799.
[17] J. Ma, Y. Xie, Y. Shi, W. Qin, B. Zhao, Y. Jin, Glucocorticoid-induced apoptosis requires FOXO3A activity, Biochem. Biophys. Res. Commun. 377 (2008) 894–898.
[18] M. Potente, C. Urbich, K. Sasaki, W.K. Hofmann, C. Heeschen, A. Aicher, R. Kollipara, R.A. DePinho, A.M. Zeiher, S. Dimmeler, Involvement of Foxo transcription factors in angiogenesis and postnatal neovascularization, J. Clin. Invest. 115 (2005) 2382–2392.
[19] A. Sunters, S. Fernandez de Mattos, M. Stahl, J.J. Brosens, G. Zoumpoulidou, C.A. Saunders, P.J. Coffer, R.H. Medema, R.C. Coombes, E.W. Lam, Foxo3a transcriptional regulation of Bim controls apoptosis in paclitaxel-treated breast cancer cell lines, J. Biol. Chem. 278 (2003) 49795–49805.
[20] A. Brunet, A. Bonni, M.J. Zigmond, M.Z. Lin, P. Juo, L.S. Hu, M.J. Anderson, K.C. Arden, J. Blenis, M.E. Greenberg, Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor, Cell 96 (1999) 857–868.
[21] N. Chapuis, S. Park, L. Leotoing, J. Tamburini, F. Verdier, V. Bardet, A.S. Green, L. Willems, F. Agou, N. Ifrah, F. Dreyfus, G. Bismuth, V. Baud, C. Lacombe, P. Mayeux, and D. Bouscary, IkappaB kinase overcomes PI3K/Akt and ERK/MAPK to control FOXO3a activity in acute myeloid leukemia. *Blood* 116 4240–50.
[22] S. Herzog, M. Reth, H. Jumaa, Regulation of B-cell proliferation and differentiation by pre-B-cell receptor signalling, Nat. Rev. Immunol. 9 (2009) 195–205.
[23] J. Bartkova, J. Lukas, M. Strauss, J. Bartek, Cyclin D3: requirement for G1/S transition and high abundance in quiescent tissues suggest a dual role in proliferation and differentiation, Oncogene 17 (1998) 1027–1037.
[24] T.H. Cheung, M.M. Yu, K.W. Lo, S.F. Yim, T.K. Chung, Y.F. Wong, Alteration of cyclin D1 and CDK4 gene in carcinoma of uterine cervix, Cancer Lett. 166 (2001) 199–206.
[25] J. Houldsworth, V. Reuter, G.J. Bosl, R.S. Chaganti, Aberrant expression of cyclin D2 is an early event in human male germ cell tumorigenesis, Cell Growth Differ. 8 (1997) 293–299.
[26] E. Sicinska, I. Aifantis, L. Le Cam, W. Swat, C. Borowski, Q. Yu, A.A. Ferrando, S.D. Levin, Y. Geng, H. von Boehmer, P. Sicinski, Requirement for cyclin D3 in lymphocyte development and T cell leukemias, Cancer Cell 4 (2003) 451–461.
[27] S. Fernandez de Mattos, A. Essafi, I. Soeiro, A.M. Pietersen, K.U. Birkenkamp, C.S. Edwards, A. Martino, B.H. Nelson, J.M. Francis, M.C. Jones, J.J. Brosens, P.J. Coffer, E.W. Lam, Foxo3a and BCR–ABL regulate cyclin D2 transcription through a STAT5/BCL6-dependent mechanism, Mol. Cell. Biol. 24 (2004) 10058–10071.
[28] R.H. Medema, G.J. Kops, J.L. Bos, B.M. Burgering, AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1, Nature 404 (2000) 782–787.