Int J Clin Exp Pathol 2016;9(10):10242-10249
www.ijcep.com /ISSN:1936-2625/IJCEP0029985

Original Article

Activation of non-classical NF-κB signaling pathway is associated with drug resistance of B cell non-Hodgkin's lymphoma

Jing Liu[1], Na Lv[1], Xin Cai[3], Kaili Zhong[2], Lei Zhou[1], Sai Huang[1], Yan Li[1], Honghao Gao[1], Wei Guan[1], Lan Yang[1], Donggang Xu[3], Hang Su[2], Li Yu[1]

[1]Department of Hematology, Chinese PLA General Hospital, Beijing, China; [2]Department of Lymphoma Therapy, Affiliated Hospital of Academy of Military Medical Sciences, Beijing, China; [3]Laboratory of Genome Engineering, Beijing Institute of Basic Medical Sciences, Beijing, China

Received March 11, 2016; Accepted July 19, 2016; Epub October 1, 2016; Published October 15, 2016

Abstract: The activation of non-classical NF-κB signaling pathway is closely related to the progression of B cell non-Hodgkin lymphoma (B-NHL). In this study we examined the status of non-classical NF-κB signaling in the peripheral blood mononuclear cells (PBMC) from 36 cases of B-NHL and 7 cases of healthy blood donators as well as the tumor tissues from another 20 cases of B-NHL. We found that the expression levels of BAFF, BAFF-R, Bcl-xL and RIPK2 in PBMC of drug-resistant and phase III/IV B-NHL were significantly higher than those of non-drug-resistant, phase I/II and normal control group ($P<0.05$). BAFF stimulated Raji cell proliferation in dose- and time-dependent manner, and upregulated mRNA and protein levels of BAFF, BAFF-R, NIK, p52, Bcl-xL and RIPK2 in a dose-dependent manner ($P<0.05$). Taken together, our results suggest that the activation of non-classical NF-κB signaling pathway is correlated with drug resistance and progression of B-NHL.

Keywords: B cell non-Hodgkin's Lymphoma (B-NHL), drug resistance, non-canonical NF-κB pathway, BAFF, Bcl-xL

Introduction

Malignant lymphoma is one of the fastest growing malignant tumors in China. At present about 84 thousand new cases of malignant lymphoma are identified every year and more than 20 thousand people die of it. B cell non-Hodgkin's lymphoma (B-NHL) is a group of heterogeneous lethal diseases derived from monoclonal expansion of malignant B cell, accounting for 60% of malignant lymphoma. With recent advances in the diagnosis and therapy, the lifespan of B-NHL patients has been gradually prolonged and some patients can even be cured. However, drug resistance of tumor cells results in invalid treatment and relapse, leading to poor prognosis in some patients. It is important to understand the mechanisms of progression and drug-resistance of B-NHL.

The expression of BAFF in peripheral blood of B-NHL patients is significantly increased, which is related to the disease's progression, severity and sensitivity to treatments [1, 2]. BAFF receptor (BAFF-R) is the specific receptor for BAFF to regulate the survival of B cells, while the non-classical NF-κB signaling pathway is the major one that is activated by BAFF/BAFF-R. Therefore, the activation of non-classical NF-κB signaling pathway by BAFF/BAFF-R may be one of the reasons for the survival, proliferation, differentiation and drug-resistance of B-NHL tumor cells [3].

In this study we aimed to investigate the correlation between non-classical NF-κB signaling activated by BAFF/BAFF-R and drug resistance in B-NHL. We detected the expression levels of components of non-classical NF-κB pathway in PBMC and lymph node tumor tissues of B-NHL patients, and human Burkitt lymphoma Raji cells.

Materials and methods

Patients and samples

The peripheral blood samples were collected from 36 patients with clinically and pathologically diagnosed B-NHL who were hospitalized in

Table 1. Clinical characteristics of B-NHL cases

Clinical characteristics		Cases (n)
Gender	Male	21
	Female	15
International Lymphoma Prognostic Index (IPI)	Score 0~2	18
	Score 3~4	18
Age*	<60	27
	≥60	9
Ann Arbor staging	Stage I/II	7
	Stage III/IV	29
Drug resistance**	Drug-resistance	9
	Non-drug resistance	27
WHO (2008) grouping for lymph tissue tumors	Diffusive large B cell lymphoma	34
	Mantle cell lymphoma	2

*: 8-74 years old with the median age of 45. **: 36 cases of B-NHL patients were all subjected to chemotherapy with R-CHOP as the major program for more than 4 cycles, and the therapeutic efficacy was evaluated by CT and other examinations, complete remission (CR) or partial remission (PR) were judged as non-drug-resistant. The therapeutic efficacy was classified into stable disease (SD) or progression of disease (PD), chemotherapy and local radiotherapy with DICE, ProMACE/CytoBOM, Gemcitabine, and other second- or third-line programs were carried out, and the patients whose conditions could not be effectively controlled were judged as drug-resistant.

the Hospital Affiliated to Chinese Academy of Military Medical Sciences from November 2010 to November 2011. The clinical characteristics of the patients were shown in **Table 1**. Seven healthy blood donators were included as the control during the same period, including four males and three females aged between 23 and 48 years old (median age of 33). 4 mL of venous blood sample was collected from B-NHL patients and healthy blood donators by using EDTA anticoagulation vacuum tubes. The samples were centrifuged at 3,000 rpm at room temperature for 10 min, the separated plasma samples were stored at -20°C for further use, and the blood sediments were immediately used for RNA extraction.

The samples from lymph node tumors were collected from 20 cases of B-NHL patients who were hospitalized at the same hospital from January 2010 to January 2011, and the fresh samples collected from operative biopsy were stored in liquid nitrogen. The study protocols were approved by the Ethics Committee of the Hospital.

Cell culture

Human Burkitt lymphoma Raji cell line was purchased from Chinese Union Cell Center, and incubated in the modified RPMI 1640 (HyClone, USA) medium containing 10% FBS (Yuanheng Jinma Biotech, China) at 37°C with 5% CO_2 and saturated humidity in a CO_2 incubator. The recombinant human cytokine BAFF was purchased from PeproTech (USA) and used for *in vitro* stimulation of Raji cell line.

Real time quantitative PCR

EASYspin whole-blood RNA fast extraction kit and EASY spin tissue/cell RNA fast extraction kit (Biomed Tech, China) were used to extract total RNA from PBMC and tissues. RNA pure ultrapure total RNA fast extraction kit (Biomed Tech, China) was used to extract total RNA from Raji cells after stimulation for 48 h with different concentrations of recombinant human BAFF (20 ng/mL, 100 ng/mL, 500 ng/mL). Primer Script Reverse Transcriptase (TaKaRa, Japan) was used for reverse transcription of 2 μg total RNA, and the resulted cDNA was used for FQ-PCR. The reaction was carried out by using the fluorescent Maxima™SYBR Green/ROX qPCR Master Mix (2×) (Fermentas, USA) on Mx3000PQPCR Systems (Agilent Technologies Stratagene Products Division, USA). The primers were synthesized by AuGCT DNA-SYN Biotech (China) with the following sequences: GAPDH 5'-ACCCAGAAGACTGTGGATGG-3' (forward) and 5'-TTCAGCTCAGGGATGACCTT-3' (reverse); BAFF 5'-AATAAGCGTGCCGTTCAG-3' (forward) and 5'-GATCCTTTTTGTATAGTTGGTGTT-3' (reverse); BAFF-R5'-TCATTCTGTCTCCGG-

Table 2. mRNA expression levels in B-NHL patients grouped by clinical stage

Group		n	BAFF	BAFF-R	Bcl-xL	RIPK2
Staging	Stage I/II	7	1.65±0.15*	1.30±0.25*	2.19±0.66*	1.68±0.31*
	Stage III/IV	29	2.11±0.41*,Δ	1.96±0.61*,Δ	2.49±0.32*,Δ	2.14±0.48*,Δ
Normal control		7	1.00±0.19	1.00±0.20	1.00±0.09	1.00±0.31

*: P<0.05, compared to control group. ΔP<0.05, compared to Stage I/II.

Table 3. mRNA expression levels in B-NHL grouped by drug-resistance

Group	n	BAFF	BAFF-R	Bcl-xL	RIPK2
Non-drug-resistance	27	1.85±0.27*	1.55±0.34*	2.30±0.37*	1.96±0.39*
Drug-resistance	9	2.52±0.36*,Δ	2.66±0.47*,Δ	2.84±0.23*,Δ	2.43±0.52*,Δ
Normal control	7	1.00±0.19	1.00±0.20	1.00±0.09	1.00±0.31

*: P<0.05, compared to control group. ΔP<0.05, compared to Non-drug-resistance.

GAATC-3' (forward) and 5'-GTGGTCACCAGTTCAGTGGA-3' (reverse); NIK 5'-TTTTCTCTGGAGGAGCAGGA-3' (forward) and 5'-GCTTTGAGAGGCCTTTGATG-3' (reverse); p52 5'-ACAGCCCTGTCCTTCAGAGA-3' (forward) and 5'-CTTCTTCCTTCTGGCCCTCT-3' (reverse); Bcl-xL 5'-GTAAACTGGGGTCGCATTGT-3' (forward) and 5'-TGGATCCAAGGCTCTAGGTG-3' (reverse); RIPK2 5'-GTTGGGACAGCACCATTTCT-3' (forward) and 5'-ATACCAGGCTGCAGACGTTC-3' (reverse). The mRNA expression of these genes was calculated by using the $2^{-\Delta\Delta Ct}$ method.

Cell proliferation assay

Raji cells were seeded in the 96-well plate (Costar, USA) in a density of 1.5×10^4 cells/well. Different concentrations of rhBAFF were added into the wells at the final concentration of 62.5 ng/mL, 125 ng/mL, 250 ng/mL and 500 ng/mL respectively, while the control group was incubated in serum-free culture medium (as blank control) and three duplicates were set for each group. MTT colorimetric tests were carried out after 24 h, 48 h and 72 h according to the standard procedures, and the absorbance was determined at 570 nm.

Western blot analysis

Raji cells were rinsed with pre-cooled 1×phosphate buffer for twice and then RIPA lysis buffer (Applygen, China) was added to extract the total protein, and protein content was determined by using Lowry method (Kaiji Biotech, China). The total protein extracted from Raji cells was loaded onto 15% SDS-PAGE and transferred to PVDF membrane. The non-specific binding sites on the membrane were blocked overnight at 4°C by using 5% defatted milk powder prepared with Tris buffer/1% Tween20 (TBS-T). The membrane was incubated with specific antibody for BAFF, BAFF-R, NIK, p52, RIPK2 (Abcam, England) and Bcl-Xl (Cell Signaling, USA) at room temperature for 2 h. The membrane was washed with TBS-T for three times and incubated with horse radish peroxidase (HRP) conjugated goat anti-rat and goat anti-rabbit antibodies at room temperature for 1 h. Finally, the membrane was developed in a dark room using SuperSignal West Femto Substrate (Thermo Scientific, USA). The house-keeping gene GAPDH (Cwbiotech, China) was used as loading control.

Statistical analysis

SPSS13.0 statistical software was used for statistical analysis. The experimental data were subjected to normality test and homogeneity test for variance. The data were expressed as mean value ± standard deviation and analyzed by t-test or ANOVA. Pearson linear correlation analysis and spearman rank correlation analysis were carried out for correlation analysis. P<0.05 indicated that the difference was statistically significant.

Results

The expression of non-classical NF-κB signaling components in PBMC of B-NHL patients

We grouped the patients according to clinical stage, and found that the expression levels of mRNAs for BAFF, BAFF-R, Bcl-xL and RIPK2 in PBMC of stage I/II and III/IV B-NHL patients were significantly higher than those in control group, and the expression levels in stage III/IV B-NHL group were significantly higher than those in stage I/II group (P<0.05, **Table 2**). In addition, the expression levels of mRNAs for BAFF, BAFF-R, Bcl-xL and RIPK2 in PBMC in drug-resistant B-NHL patients were significantly higher than those in control group and non-drug-resistant B-NHL patient group (P<0.05, **Table 3**).

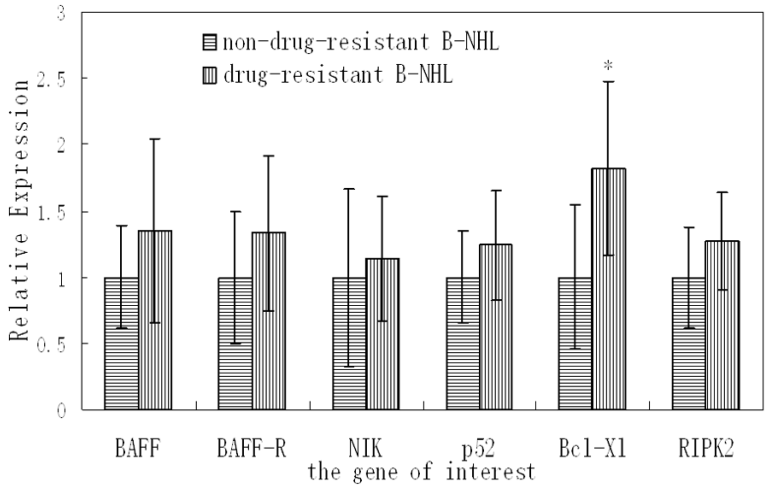

Figure 1. The expression levels of mRNAs of indicated genes in the tumor tissues from B-NHL patients with different drug-tolerances. *P<0.05.

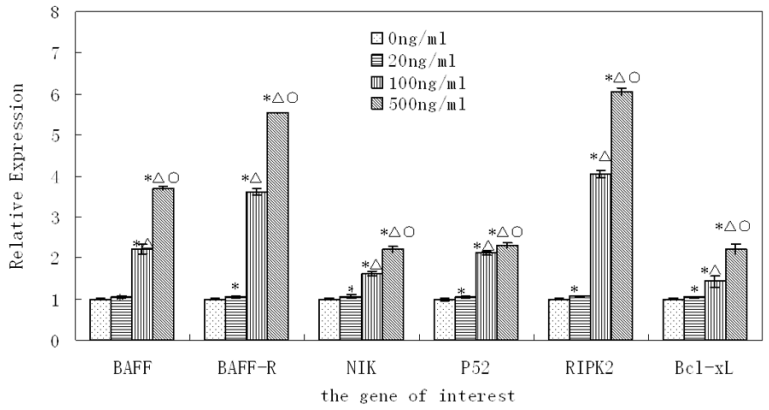

Figure 2. The expression levels of mRNAs of indicated genes in Raji cells treated with different concentration of recombinant human BAFF.

Next we performed correlation analysis on the expression levels of mRNAs. Pearson linear correlation analysis showed that the r values for the correlation between two genes grouped by clinical stage were as follows: BAFF and BAFF-R 0.849, BAFF and Bcl-xL 0.924, BAFF and RIPK2 0.866, BAFF-R and Bcl-xL 0.862, BAFF-R and RIPK2 0.821, Bcl-xL and RIPK2 0.878 (P<0.001 for all). The r values for the correlation between two genes grouped by drug-resistance were as follows: BAFF and BAFF-R 0.757, BAFF and Bcl-xL 0.774, BAFF and RIPK2 0.753, BAFF-R and Bcl-xL 0.683, BAFF-R and RIPK2 0.652, Bcl-xL and RIPK2 0.649 (P<0.001 for all). These results indicated significant positive correlation among the mRNA expression of these genes.

Spearman rank correlation analysis was carried out to investigate the correlation between the expression levels of mRNAs for these genes and the progression of disease. The r values for the correlation among BAFF, BAFF-R, Bcl-xL and RIPK2 in control, stage I/II and stage III/IV groups were 0.828, 0.753, 0.828, 0.811, respectively (P<0.001 for all). The r values for the correlation among BAFF, BAFF-R, Bcl-xL and RIPK2 in control, non-drug-resistant and drug-resistant groups were 0.855, 0.811, 0.834, 0.713, respectively (P<0.001 for all). These data indicated that mRNA expression of these genes was closely related to the progression and drug resistance of B-NHL.

The expression of non-classical NF-κB signaling components in B-NHL tissues

B-NHL patients were grouped by gender and IPI scores, and the mRNA expression levels of BAFF, BAFF-R, NIK, p52, Bcl-xL and RIPK2 of male B-NHL patient group and with IPI score 0~2 group were not statistically significantly different from those in the female and with IPI score 3~4 group.

The expression level of Bcl-xL mRNA in drug-resistant group was significantly higher than that in non-drug-resistant group (P<0.05). However, the expression levels of mRNAs for BAFF, BAFF-R, NIK, P52 and RIPK2 in drug-resistant group showed increases compared to those in non-drug-resistant group but without significant differences (**Figure 1**).

The expression of non-classical NF-κB signaling components in Raji cells

Raji cells were stimulated with different concentration of recombinant human BAFF, and the expression levels of mRNAs for BAFF, BA-

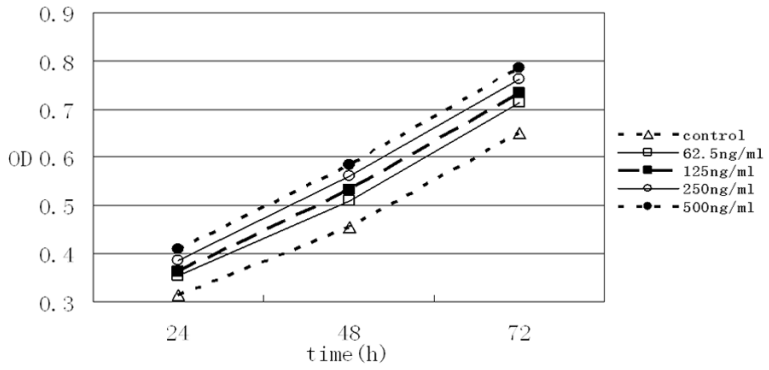

Figure 3. rhBAFF stimulates the proliferation of Raji cells. MTT assay of Raji cells treated with rhBAFF at the indicated concentration and time.

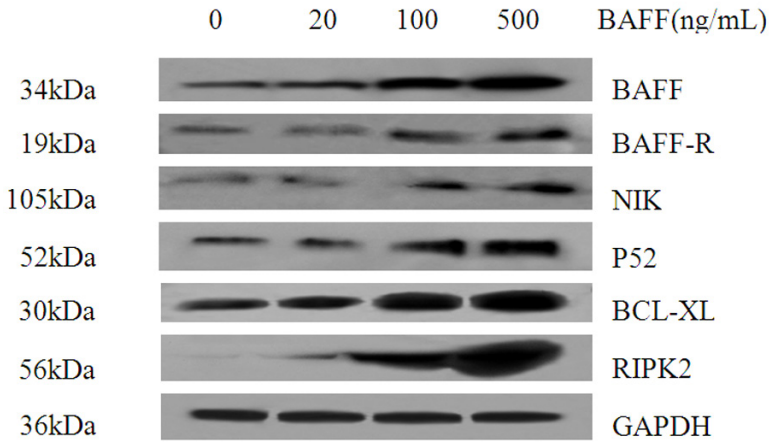

Figure 4. rhBAFF activates non-classical NF-κB signaling in Raji cells. Western blot analysis of the expression levels of the indicated proteins in Raji cells after stimulation with different concentration of rhBAFF. GAPDH was loading control.

FF-R, NIK, p52, Bcl-xL and RIPK2 were significantly increased compared to untreated control ($P<0.05$). The differences of mRNA expression level for these genes in the groups treated with 20, 100 and 500 ng/mL of recombinant human BAFF (rhBAFF) were significant ($P<0.05$, **Figure 2**).

Next we performed Pearson linear correlation analysis on the expression levels of mRNAs in Raji cells treated with different concentration of rhBAFF. The r values for the correlation among BAFF, BAFF-R, Bcl-xL, RIPK2 and the concentration of rhBAFF were 0.972, 0.950, 0.971, 0.953 ($P<0.001$ for all). These results indicated significant positive correlation among the mRNA expression of these genes and the concentration of rhBAFF.

BAFF promotes Raji cell proliferation

Compared to control group, the OD values in different groups after stimulation with different concentration of rhBAFF for 24 h, 48 h and 72 h showed increases to different extents, and significant differences could be detected at different concentration and different time point (**Figure 3**), indicating that BAFF promoted the proliferation of Raji cells in dose- and time- dependent manner.

BAFF stimulates the activation of non-classical NF-κB signaling in Raji cells

Western blot analysis showed that the protein levels of BAFF, BAFF-R, NIK, P52, Bcl-xL and RIPK2 were increased in Raji cells after treatment with BAFF in a dose-dependent manner (**Figure 4**).

Discussion

Malignant lymphomas mainly develop in lymph nodes and lymph tissues. B-NHLs are a group of heterogonous malignant lethal diseases due to monoclonal proliferation of malignant B cells. Previous studies confirmed that some signaling pathways such as PI3K-Akt, Notch, Hh are involved in the development of B-NHL [5-9]. This study aimed to explore the correlation between drug-resistance of B-NHL and non-classical NF-κB signaling pathway. We found higher mRNA expression levels of the components of non-classical NF-κB signaling in peripheral blood and tumor tissues from B-NHL patients, and demonstrated that BAFF stimulated the proliferation and the activation of non-classical NF-κB pathway in Raji cells.

BAFF is also named as B lymphocyte stimulating factor (BLys) [10-12], which can specifically stimulate the survival, maturation, proliferation and differentiation of B cells [13-15]. B cells

were almost completely absent from follicles and marginal zones in BAFF-defective mice [16], while injection with recombinant Fc-BAFF or protected B cell compartments in marginal zone and follicles in BAFF-defective mice [17]. In contrast, B cell compartments in marginal zone of BAFF transgenic mice were significantly enlarged [18]. Animal studies showed that both the defect and overexpression of BAFF could lead to immunological imbalance and subsequently induce many diseases [19, 20]. Malignant B cells can secrete BAFF, and BAFF level in the peripheral blood of patients with B cell malignant tumors is related to tumor progression and sensitivity to treatments [1, 2]. Therefore, endogenous BAFF level may be closely related to the initiation, progression and drug-resistance of B-NHL.

BAFF regulates the proliferation, activation and function of B cells by binding to corresponding receptors [21, 22], among which BAFF-R is the specific receptor to regulate the survival of B cells. The non-classical NF-κB signaling pathway is one of the major pathways mediated by BAFF/BAFF-R, which has important roles in maintaining the functions of B cells [23]. Therefore, the activation of non-classical NF-κB signaling pathway may be one of the major reasons for drug-resistance in B-NHL [3]. Drug resistance in B-NHL is correlated with highly activated NF-κB in tumor cells, the over-expression of downstream targets Bcl-xL, Bcl-2 and other anti-apoptotic factors, the inhibition of tumor cell apoptosis, and the promotion of survival, maturation and proliferation of tumor cells [24]. Novak et al. found that BAFF mutation was correlated with the incidence of follicular lymphoma (FL) and serum BAFF concentration in B-NHL patients [25]. In this study, we found that the mRNA expression levels of BAFF, BAFF-R and Bcl-xL in PBMC from different B-NHL patient groups were significantly higher than those in control group. In addition, the expression levels of BAFF, BAFF-R and Bcl-xL were related to the stage and drug resistance of B-NHL, but not with the gender, IPI score and age of patients. These results indicate that non-classical NF-κB signaling pathway is correlated with the progression and drug-resistance of B-NHL. The mRNA expression levels of BAFF, BAFF-R, NIK, p52 and Bcl-xL were also detected in B-NHL tumor tissues. Bcl-xL mRNA expression level in drug resistant group was significantly higher than that in non-drug resistant group. The mRNA expression levels of BAFF, BAFF-R, NIK and p52 in drug resistant group showed increasing tendency compared to non-drug resistant group, but had no significant differences. These may be attributed to fewer cases and higher variation coefficients for the detection of gene expression. Furthermore, the clinical treatment programs were different, ranging from radiotherapy to chemotherapy.

RIPK2, also named as RIP2, CARDIAK and RICK, belongs to the RIP kinase family. and is a key mediator of non-classical NF-κB signaling pathway [26, 27]. In this study, we found that the expression levels of RIPK2 mRNA in PBMC of B-NHL patients were significantly higher than those in control group, and the expression levels in drug resistant group were significantly higher than in non-drug-resistant group, consistent with the changing tendency in mRNA expression of BAFF, BAFF-R, Bcl-xL and other non-classical NF-κB signaling molecules. In tumor samples the expression levels of RIPK2 mRNA in drug resistant group showed an increasing tendency compared to non-drug-resistant group, further indicating that RIPK2 is involved in non-classical NF-κB signaling. Furthermore, using Raji cells as the experimental model, we showed that BAFF could promote the proliferation of Raji cells in a dose and time dependent manner, accompanied by increased mRNA levels of BAFF, BAFF-R, NIK, p52 and Bcl-xL. Western blot analysis confirmed that BAFF increased the protein levels of BAFF-R, NIK, p52 and Bcl-xL in a dose-dependent manner. BAFF also upregulated the mRNA and protein expression of RIPK2, suggesting that RIPK2 participates in the non-classical NF-κB signaling in Raji cells.

In summary, we found that non-classical NF-κB signaling was closely related to drug resistance and disease progression of B-NHL. These results provided new clues for further understanding of the mechanisms for the initiation, progression and drug resistance of B-NHL, and help develop effective therapeutic approaches for B-NHL.

Acknowledgements

This study was supported by grants from National Natural Science Foundation of China (No. 81071945, 30873030, 81071928) and

Doctorial Innovation Foundation of People's Liberation Army Medical University (No. B14-009).

Disclosure of conflict of interest

None.

Address correspondence to: Li Yu, Department of Hematology, Chinese PLA General Hospital, Beijing 100853, China. E-mail: liyu301@vip.163.com

References

[1] Dalle S, Dupire S, Brunet-Manquat S , Reslan L, Plesa A, Dumontet C. In vivo model of follicular lymphoma resistant to rituximab.Clin Cancer Res 2009; 5: 851-857.

[2] Novak AJ, Grote DM, Stenson M, Ziesmer SC, Witzig TE, Habermann TM, Harder B, Ristow KM, Bram RJ, Jelinek DF, Gross JA, Ansell SM. Expression of Blys and its receptors in B-cell non-Hodgkin lymphoma:correlation with disease activity and patient outcome. Blood 2004; 104: 2247- 2253.

[3] Ansell SM, Novak AJ, Ziesmer S, Price-Troska T, LaPlant B, Dillon SR, Witzig TE. Serum BLyS levels increase after rituximab as initial therapy in patients with follicular Grade 1 non-Hodgkin lyrnphoma. Am J Hematol 2009; 84: 71-73.

[4] Mahalingam D, Medina EC, Esquivel JA 2nd, Espitia CM, Smith S, Oberheu K, Swords R, Kelly KR, Mita MM, Mita AC, Carew JS, Giles FJ, Nawrocki ST. Vorinostat enhances the activity of temsirolimus in renal cell carcinoma through suppression of survivin levels. Clin Cancer Res 2010; 16: 141-153.

[5] Uddin S, Hussain AR, Siraj AK, Manogaran PS, Al-Jomah NA, Moorji A, Atizado V, Al-Dayel F, Belgaumi A, El-Solh H, Ezzat A, Bavi P, Al-Kuraya KS. Role of phosphatidylinositol 3'- kinase /AKT pathway in diffuse large B-cell lymphoma survival. Blood 2006; 108: 4178-4186.

[6] Lee SY, Kumano K, Nakazaki K, Sanada M, Matsumoto A, Yamamoto G, Nannya Y, Suzuki R, Ota S, Ota Y, Izutsu K, Sakata-Yanagimoto M, Hangaishi A,Yagita H, Fukayama M, Seto M, Kurokawa M, Ogawa S, Chiba S. Gain-of-function mutations and copy number increases of Notch2 in diffuse large B- cell lymphoma. Cancer Sci 2009; 100: 920-926.

[7] Zweidler-McKay PA, He Y, Xu L, Rodriguez CG, Karnell FG, Carpenter AC, Aster JC, Allman D, Pear WS. Notch signaling is a potent inducer of growth arrest and apoptosis in a wide range of B-cell malignancies. Blood 2005; 106: 3898-3906.

[8] Kim JE, Singh RR, Cho-Vega JH, Drakos E, Davuluri Y, Khokhar FA, Fayad L, Medeiros LJ, Vega F. Sonic hedgehog signaling proteins and ATP-binding cassette G2 are aberrantly expressed in diffuse large B-Cell lymphoma. Mod Pathol 2009; 22: 1312-1320.

[9] Singh RR, Kim JE, Davuluri Y, Drakos E, Cho-Vega JH, Amin HM, Vega F. Hedgehog signaling pathway is activated in diffuse large B-cell lymphoma and contributes to tumor cell survival and proliferation. Leukemia 2010; 24: 1025-1036.

[10] Mackay F, Schneide P. Cracking the BAFF code. Nat Rev Immunol 2009; 9: 491-502.

[11] Lwin T, Crespo LA, Wu A, Dessureault S, Shu HB, Moscinski LC, Sotomayor E, Dalton WS, Tao J. Lymphoma cell adhesion-induced expression of B cell-activating factor of the TNF family in bone marrow stromal cells protects non-Hodgkin's B lymphoma cells from apoptosis. Leukemia 2009; 23: 170-177.

[12] Yang J, Pospisil R, Ray S, Milton J, Mage RG. Investigations of a rabbit (Oryctolagus cuniculus) model of systemic lupus erythematosus (SLE), BAFF and its receptors. PLoS One 2009; 4: e8494.

[13] Treml JF, Hao Y, Stadanlick JE, Cancro MP. The BLyS family: toward a molecular understanding of B cell homeostasis. Cell Biochem Biophys 2009; 53: 1-16.

[14] Moisini I, Davidson A. BAFF: a local and systemic target in autoimmune diseases. Clin Exp Immunol 2009; 158: 155-163.

[15] Liu Z, Davidson A. BAFF inhibition: A new class of drugs for the treatment of autoimmunity. Exp Cell Res 2011; 317: 1270-1277.

[16] Schiemann B, Gommerman JL, Vora K, Cachero TG, Shulga-Morskaya S, Dobles M, Frew E, Scott ML. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. Science 2001; 293: 2111- 2114.

[17] Swee LK, Tardivel A, Schneider P, Rolink A. Rescue of the mature B cell compartment in BAFF-deficient mice by treatment with recombinant Fc-BAFF. Immunol Lett 2010; 131: 40-48.

[18] Batten M, Groom J, Cachero TG, Qian F, Schneider P, Tschopp J, Browning JL, Mackay F. BAFF mediates survival of peripheral immature B lymphocytes. J Exp Med 2000; 192: 1453-1466.

[19] Zhou L, Zhong R, Hao W, Wang H, Fan X, Zhang L, Mi Q. Interleukin-10 and interferon-gamma up-regulate the expression of B-cell activating factor in cultured human promyelocytic leukemia cells. Exp Mol Pathol 2009; 87: 54-58.

[20] [20]Krivosikova M, Dallos T, Maslinski W, Buc M. B cell activating factor, its role in autoimmunity, and targeting in autoimmune diseases. Bratisl Lek Listy 2009; 110: 137-145.

[21] Bossen C, Schneider P. BAFF, APRIL and their receptors: structure, Function and signaling. Semin Immunol 2006; 18: 263-275.
[22] Daniel S, Anikó H, Judit P. Cooperation between signalling pathways leading to survival, proliferation or death of B cells. Ann Rheum Dis 2011; 70: A15.
[23] Fu L, Lin-Lee YC, Pham LV, Tamayo AT, Yoshimura LC, Ford RJ. BAFF-R promotes cell proliferation and survival through interaction with IKKbeta and NF-kappaB/c-Rel in the nucleus of normal and neoplastic B-lymphoid cells. Blood 2009; 113: 4627-4636.
[24] Jazirehi AR, Vega MI, Bonavida B. Development of rituximab-resistant lymphoma clones with altered cell signaling and cross-resistance to chemotherapy. Cancer Res 2007; 67: 1270-81.
[25] Novak AJ, Slager SL, Fredericksen ZS, Wang AH, Manske MM, Ziesmer S, Liebow M, Macon WR, Dillon SR, Witzig TE, Cerhan JR, Ansell SM. Genetic variation in B-cell-activating factor is associated with an increased risk of developing B-cell non-Hodgkin lymphoma. Cancer Res 2009; 69: 4217-4224.
[26] Hasegawa, M, Fujimoto Y, Lucas PC, Nakano H, Fukase K, Núñez G, Inohara N. A critical role of RICK/RIP2 polyubiquitination in Nod-induced NF-kappaB activation. EMBO J 2008; 27: 373-383.
[27] Du X, Jiang S, Liu H, Xin X, Li J, Geng M, Jiang H. MS80, a novel sulfated polysaccharide, inhibits CD40-NF-kappaB pathway via targeting RIP2. Mol Cell Biochem 2010; 337: 227-285.