Article published online: 2021-06-03

Original Article

FoxO3a Gene Down-regulation in Pathogenesis of Pediatric Acute Lymphoblastic Leukemia

Malihe Mirzaie, Mahboobeh Nasiri, Mehran Karimi[1], Majid Yavarian[1], Arghavan Kavosi[2]

Department of Biology, Islamic Azad University, Arsanjan, [1]Hematology Research Center, Shiraz University of Medical Sciences, Shiraz, [2]Department of Cellular and Molecular Biology, Faculty of Advanced Sciences and Technology, Islamic Azad University, Tehran, Iran

Abstract

Introduction: Acute lymphoblastic leukemia (ALL) is the most common malignancy found in the pediatrics with the peak prevalence between the ages of 2 and 5 years. The constitutive activation of PI3K/AKT pathway inhibits the tumor-suppressor role of *FoxO3a* (a member of the forkhead class O [FoxO] transcription factor family) in a variety of cancers and leads to tumorigenesis. This study aims to investigate the expression of *FoxO3a* in three different stages of pediatric ALL in mRNA level. **Subjects and Methods:** In this case-control study, 70 patients with childhood ALL and 70 healthy age- and gender-matched as the control group were enrolled. Real-time quantitative RT-polymerase chain reaction (qRT-PCR) was used to detect the mRNA expression level of *FoxO3a* in children with different stages of ALL and healthy children as a control group. **Results:** Data showed that the expression of *FoxO3a* mRNA was lower in newly diagnosed ALL patients compared to controls ($P < 0.0001$), maintenance ($P = 0.0342$), and relapse ($P = 0.0006$) groups, while no difference was observed between other groups. In addition, T-ALL patients showed decreased expression of *FoxO3a* compared to Pre-B ALL ones ($P < 0.0001$). **Conclusion:** The study results suggest that *FoxO3a* plays a tumor-suppressor role in ALL. Thus, its up-regulation seems to be a plausible therapeutic strategy for this type of tumor.

Keywords: *FoxO3a, gene expression, pediatric acute lymphoblastic leukemia, PI3K/AKT pathway*

Introduction

Acute lymphoblastic leukemia (ALL), one of the four main types of leukemia, is a clonal hematological disorder which occurs due to an uncontrolled proliferation of undifferentiated or poorly differentiated lymphocytes in bone marrow that quickly spread into the blood.[1] ALL can occur at any age. However, it is the most common pediatric malignancy diagnosed in children under the age of 20 years.[2] ALL is divided into two main subtypes based on tumor cell immunophenotype and histology: Lymphoblasts of B-and T-lineage.[3] Since ALL is a multifactorial disease, it can arise from interactions between exogenous or endogenous exposures, genetic susceptibility, and chance.[2,4] Genetically, fundamental changes in proto-oncogenes and tumor-suppressor genes can cause leukemia, since these changes alter key regulatory processes, including self-renewal, proliferation, differentiation, and apoptosis in target cells.[5]

Deregulation of the PI3K (Phosphoinositide 3-kinase)/Akt (Protein kinase B) signaling pathway is involved in tumorigenesis and chemotherapeutic resistance, and it has been investigated in a variety of cell lines.[6] This pathway triggers by phosphorylation cascade in which Akt is phosphorylated and leads to phosphorylation and activation or inactivation of several important genes.[7] *FoxO3a*, a member of the Forkhead family of transcription factors (FoxO1, *FoxO3a*, FoxO4, and FoxO6 in human), is one of the direct downstream targets of the PI3K/Akt pathway, regulating the cell cycle, cell growth, apoptosis, DNA damage responses, and angiogenesis.[6,8] *FoxO3a* functions as a tumor-suppressor gene and expresses in various tissues such as B- and T-lineage cells.[9] This gene transcriptionally activates various target genes including *P27*, *P21*, *P130*, and *cyclin D1/2* (cell cycle regulation),[10] *Bim*, *Fas ligand*, *TRAIL*, *Puma* (apoptosis),[11] and *GADD45a* (DNA repair).[12] Another important role of *FoxO3a*, which has been reported recently, is the modulation of the response to physiologic oxidative stress leading to

How to cite this article: Mirzaie M, Nasiri M, Karimi M, Yavarian M, Kavosi A. *FoxO3a* gene down-regulation in pathogenesis of pediatric acute lymphoblastic leukemia. Indian J Med Paediatr Oncol 2019;40:381-5.

Submitted: 29-Sep-2017
Accepted in Revised Form: 18-Apr-2018
Published: 04-Dec-2019

Address for correspondence:
Asst Prof. Mahboobeh Nasiri, Department of Biology, Islamic Azad University, Arsanjan Branch, Arsanjan, Iran.
E-mail: nasiri@jaua.ac.ir

Access this article online

Website: www.ijmpo.org

DOI: 10.4103/ijmpo.ijmpo_203_17

Quick Response Code:

maintenance of the hematopoietic stem cell (HSC) pool.[13] Phosphorylation of *FoxO3a* by Akt prevents its nuclear translocation, thereby activation of these target genes are inhibited.[14] Hyperactivation of PI3K/Akt pathway has been investigated in various types of cancers and leukemia is not the exception.[15] Due to hyperactivated PI3K/Akt pathway, *FoxO3a* is exported from the nucleus to the cytoplasm, where it interacts with 14-3-3 proteins, resulting in its proteasomal degradation and loss of function as a transcription factor.[16] Furthermore, it has been recognized that, in B-and T-cell lines, overexpression of *FoxO3a* leads to cell cycle arrest in G1 phase and induction of P27 (cell cycle inhibitor protein) and Fas ligand and Bim (pro-apoptotic molecules) which in turn trigger apoptosis.[17]

These findings strongly suggest that the loss of the transcriptional function of *FoxO3a* by hyperactivation of PI3K/Akt pathway is involved in the pathophysiology of leukemia. However, to the best of our knowledge, few reports have been published concerning the role of *FoxO3a* in pediatric ALL. Since the expression profile of *FoxO3a* in different stages of pediatric ALL has not yet been assessed, we aim to analyze the mRNA expression level of *FoxO3a* in three different groups of children in different stages of ALL in the south Iranian population.

Subjects and Methods

Patients' characteristics and sample collection

In this case-control study, seventy patients diagnosed with childhood ALL and 70 healthy age- and gender-matched (±5 years) children without a history of any malignancies as the control group were enrolled. Healthy controls were selected from the same geographic area, and none of them were relative to cases. Patients ranged in age from 1 to 20 years old. Based on the stage of malignancy, patients were divided into three categories: (a) 30 newly diagnosed ALL patients before therapy; (b) 20 ALL patients in the maintenance phase of chemotherapy; and (c) 20 ALL patients who showed relapse 1 year after they acquired the last treatment. The cases were recruited from referral Oncology Hospital in Shiraz, southern Iran between February 2015 and August 2015. The diagnosis was confirmed using immunology and cytogenetic tests as well as monitoring the morphology of the bone marrow cells. Patients who met the following criteria were excluded from sampling; (a) age more than 20 years and under 1 year; (b) The presence of other hematological disorders or history of other malignancies; and (c) Patients with other stages of treatment.

A designed questionnaire was administered for subjects to collect information, including parental alcohol consumption, cigarette smoking status, family history of blood disorders, and other cancers. The written informed consent was obtained from parents of all children who participated in the study before sampling. This study was approved by the Ethics Committee of Islamic Azad University, Arsanjan Branch (Arsanjan, Iran).

RNA extraction and real-time polymerase chain reaction analysis

Real-time polymerase chain reaction (PCR) was performed to determine the expression level of *FoxO3a* gene, as a candidate gene involving in the pathogenesis of ALL. Total RNA was isolated from Fresh blood samples using TRIzol solution (Invitrogen, USA) according to the manufacturer's protocol. RNase-Free DNase I was used to remove DNA contamination. RNA quality and concentration was assessed by absorbency at 260 nm and 280 nm using a Nanodrop spectrophotometer (ND-1000, Thermo Scientific, USA) to normalized the concentration of all RNA samples. Then, cDNA was synthesized from 5 ng of total RNA using the RevertAid first-strand cDNA synthesis kit (Thermo Scientific Fermentas, USA) following the manufacturer's instructions. Primer pairs were designed by Allele ID v7.8 software. Real-time PCR was performed using the Rotor-Gene Q 2plex HRM platform, real-time PCR system (Corbet life science) using the following primers for *FoxO3a* gene; Forward, 5'-CGGACAAACGGCTCACTCT-3' and reverse, 5'-GGACCCGCATGAATCGACTAT-3'; and forward, 5'-CCCGAAACGCCGAATATAAT-3' and reverse, 5'-CTGGACTGTTCTTCACTCTTG-3' for TATA-binding protein gene (*TBP*) as an endogenous control gene. Each 15 µl reaction volume contained 7.5 µl of 2X SYBR Green master mix (Invitrogen, USA), 1 µl of cDNA, and 0.4 µl (10 pm) of each pair of oligonucleotide primers. All reactions were done in duplicate. The cycling parameters began with an initial step of 95˚C for 10 min followed by 35 cycles of 95°C for 25 s, 56°C for 20 s and 72°C for 10 s; then, a melting curve analysis was performed. The threshold cycle (CT) values were measured during the exponential amplification phase by Rotor-gene Q sequence detection system. The expression data were normalized to the expression level of the housekeeping gene, *TBP* and the comparative threshold cycle ($2^{-\Delta\Delta CT}$) method was used to analyze the data.

Statistical analysis

Probable risk factors for ALL and other categorical variables were compared between ALL patients (cases) and healthy children (controls) using Chi-square test (version 16.0, SPSS Inc., Chicago, IL, USA). The expression data expressed as mean ± standard deviation, for comparisons between two groups, unpaired 2-tailed students *t*-test; and to compare more than two groups, ANOVA followed by the Bonferroni multiple comparison *post hoc* tests were applied using GraphPad Prism7 software (La Jolla, USA). The value of $P < 0.05$ was considered statistically significant.

Results

The frequencies of demographic and clinical features of ALL cases and controls are presented in Table 1. The cases and controls were matched for age and gender (P = 0.12 and P > 0.99, respectively). Significantly, greater number of parents in case group were identified with smoking (P = 0.001) and drinking habit (P = 0.033); moreover, the history of blood disorders and other malignancies in the first- and second-degree relatives (P = 0.025), as well as other malignancies in first- to third-degree relatives were higher in cases compared to controls (P = 0.002). Immunophenotyping revealed that 51.4% of the ALL patients were Pre-B ALL, 17.1% were B-ALL and 31.4% were T-ALL. The proportions of patients based on the lymphoblast type were as follows: 74.3% L1, 18.6% L2, and 7.1% L3 types.

There was no significant difference in the frequency of blood groups between the cases and control subjects, but the frequency of B and O blood groups was higher among cases compared to the control group [Table 2].

Quantitative RT-PCR were performed for expression profiling of *FoxO3a* gene in blood samples of 30 newly diagnosed ALL patients, 20 patients in the maintenance phase of treatment, 20 relapse ALL patients and 70 healthy controls. The *FoxO3a* expression was significantly lower in newly diagnosed ALL patients compared to the control group (3.5-fold down-regulation, P < 0.0001), the maintenance group (2-fold down-regulation, P = 0.0342), and the relapse group (3.5-fold down-regulation, P = 0.0006), but the differences in expression level of *FoxO3a* were not statistically significant between control/maintenance, control/relapse, and maintenance/relapse groups [Figure 1].

In further analysis, the mRNA expression level of *FoxO3a* was evaluated in comparison to each two groups of ALL regarding the types of the lymphoblasts. The mRNA expression level of *FoxO3a* was significantly lower in T-ALL patients compared to the Pre-B ALL ones (3.4-fold down-regulation, P < 0.0001). No significant difference was seen between T-ALL/B-ALL and/or B-ALL/Pre-B ALL [Figure 2].

Table 1: Demographic characteristics of the study groups

Variables	Cases (n=70), n (%)	Controls (n=70), n (%)	P
Age (years)			
≤5	22 (31.4)	31 (44.3)	0.12*
>5	48 (68.6)	39 (55.7)	
Gender			
Male	41 (58.6)	41 (58.6)	>0.99
Female	29 (41.4)	29 (41.4)	
Parental smoking status			
Never	41 (58.6)	59 (84.3)	0.001
Ever	29 (41.4)	11 (15.7)	
Parental drinking status			
Never	56 (80)	65 (92.9)	0.033
Ever	14 (20)	5 (7.1)	
Blood cancers or disorders history (1 and 2 degree family)			
Negative	54 (77.1)	64 (91.4)	0.025
Positive	16 (22.9)	6 (8.6)	
Other cancer history (1-3 degree family)			
Negative	47 (67.1)	63 (90)	0.002
Positive	23 (32.9)	7 (10)	
Immunophenotype			
Pre-B ALL	36 (51.4)	-	
B-ALL	12 (17.1)	-	
T-ALL	22 (31.4)	-	
Lymphoblast type			
L1	52 (74.3)	-	
L2	13 (18.6)	-	
L3	5 (7.1)	-	

*Pearson Chi-square test, P<0.05 considered significant. Frequency percentages are in parenthesis. ALL – Acute lymphoblastic leukemia

Table 2: Distribution of blood group frequencies in patients and controls

Types	Blood groups							
	A^+	A^-	B^+	B^-	O^+	O^-	AB^+	AB^-
Controls (%)	18 (25.7)	9 (12.9)	8 (11.4)	1 (1.4)	19 (27.1)	3 (4.3)	9 (12.9)	3 (4.3)
Cases (%)	13 (18.6)	2 (2.9)	19 (27.1)	3 (4.3)	25 (35.7)	3 (4.3)	3 (4.3)	2 (2.9)

P: 0.065, Pearson χ^2; P<0.05 considered significant

Discussion

Tumor progression depends on different types of factors including heterogeneous interactions of genetic and environmental stimuli.[18] The constitutive activation of multiple signal transduction pathways enhances the survival and proliferation of leukemic cells, among them, PI3K/AKT signaling pathway has a critical role in tumor development which target *FoxO3a* tumor-suppressor gene.[19] Since this transcription factor regulates a wide range of target genes implicated in cell-cycle inhibition, resistance to oxidative stress, and apoptosis, loss of its functions has been observed in a number of human cancers and leads to tumorigenesis.[20] Conditional deletion of *FoxO1*, *FoxO3a*, and *FoxO4* in the adult hematopoietic system leads to loss of HSC maintenance.[21] It has been found that *FoxO3a* deficient mice faced with impaired long-term reconstruction of HSCs, Moreover, it was shown that the number of HSC in aging mice whose *FoxO3a* gene was knocked out was increased in comparison with wild-type littermate controls.[22] On the other hand, FoxO transcription factors have a key role in lineage development,

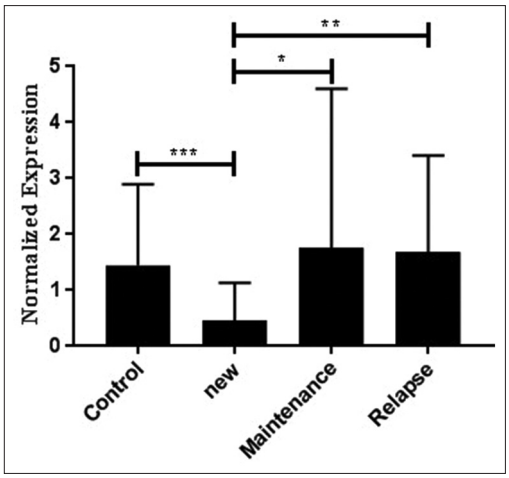

Figure 1: Comparison of the mRNA expression level between controls, new cases, and maintenance and relapse cases using quantitative reverse transcriptase-polymerase chain reaction. The expression level of *FoxO3a* gene was significantly lower in newly diagnosed cases compared to controls [* for the *P* value < o.oo1, while * and ** for *P* < 0.05] (*P* < 0.0001) and other cases in different stage of treatment (*P* < 0.05)**

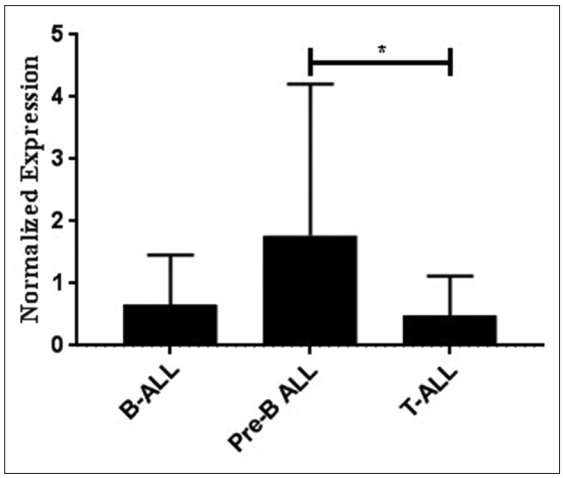

Figure 2: Intercomparsion of *FoxO3a* gene expression between pre-B, B-and T-ALL using quantitative reverse transcriptase-polymerase chain reaction. *FoxO3a* gene expression was significantly lower in T-ALL patients compared to pre-B ALL cases* (*P* < 0.0001)

in this case, conditional deletion of *FoxO1*, *FoxO3,* and *FoxO4* leads to increasing the level of myeloid cells and decreasing the number of peripheral blood lymphocytes.[21] Mutation of *FoxO3a* in hematopoietic progenitor cells of mice results in decreased in the formation of both myeloid and erythroid colonies supporting its role in lineage development.[23] It is revealed that the stronger PI3K activity in acute myeloid leukemia (AML) cells was associated with a higher rate of spontaneous proliferation of the cells;[24] Furthermore, it has been made obvious that the tumor suppressive function of *FoxO3a* is inactivated in AML cells due to its cytoplasmic localization.[25] It has been revealed that inactivation of *FoxO3a* leads to B-cell resistance to apoptosis and suggests that it might constitute a therapeutic target in this disease.[26] Down-regulation of *FoxO3a* gene has been seen in several types of tumors, including breast, ovarian, prostate, and gastric cancers, besides it has been shown that over-expression of *FoxO3a* inhibits cell proliferation and prevents tumor progression in these types of cancers.[27-30] Most of these studies were carried out in nonlymphoid cell lines, and hence, its relevance to B-and T-cells is not clear. This pursued us to analyze the mRNA expression level of *FoxO3a* gene in both B-ALL and T-ALL patients in three different stages. We found, using real-time PCR (qRT-PCR), a decreased in the expression of *FoxO3a* in the newly diagnosed ALL patients in comparison with control, maintenance, and relapse groups. Between-group comparisons revealed no statistical differences, suggesting that the chemotherapy affects the expression of *FoxO3a*. Concerning the ALL type (Pre-B ALL, B-ALL and T-ALL), T-ALL patients showed 3.4-fold down-regulation in the mRNA expression level of *FoxO3a* compared to the Pre-B ALL patients. These results are consistent with the findings of Ausserlechner *et al*. who reported that therapy-resistant T-ALL patients showed cytoplasmic localization of FoxO3a and also it is indicated that T-ALL cells in these patients inactivate *FoxO3a* to escape apoptosis induction by *TRAIL* and *NOXA* genes.[15] On the other hand, constitutive hyperactivation of PI3K/AKT pathway due to decreased PTEN activity has been seen in adult B-cell ALL cells.[31] Dewar *et al.* have shown that *FoxO3a* is a good biomarker for BCR-ABL-mediated leukemogenesis. They also found proteasomal inhibition of *FoxO3a* by bortezomib may be a promising therapeutic option in Philadelphia-positive ALL, where *FoxO3a* is down-regulated.[32] Tang *et al*. in 2016 showed that butien inhibited cell proliferation and induced cell cycle arrest in ALL via *FoxO3a*/p27kip1 pathway.[33] These reports indicate that maintenance of proper *FoxO3a* levels is critical in preventing leukemogenesis.

Conclusion

FoxO3a was found for the first time to have decreased expression in newly diagnosed pediatric ALL, and thus, it plays a tumor suppressor role in ALL. Increasing the activity of the *FoxO3a* transcription factor seems to be a plausible new therapeutic strategy for this type of tumor. Our result suggests that the *FoxO3a* can be a potential therapeutic target in pediatric ALL.

Acknowledgment

The authors express their special thanks to specialist Dr. Zarei far (head of Amir Oncology Hospital) and their staffs for their kind collaboration in blood sampling.

Financial support and sponsorship

This study was financially supported by Islamic Azad University, Arsanjan Branch, Arsanjan, Iran.

Conflicts of interest

There are no conflicts of interest.

References

1. Wang Y, Zhou L, Chen J, Li J, He L, Wu P, *et al.* Association of the 3'UTR *FOXO3a* polymorphism rs4946936 with an increased risk of childhood acute lymphoblastic leukemia in a Chinese population. Cell Physiol Biochem 2014;34:325-32.
2. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet 2013;381:1943-55.
3. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, *et al.* The 2016 Revised to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127:2391-405.
4. Belson M, Kingsley B, Holmes A. Risk factors for acute leukemia in children: A review. Environ Health Perspect 2007;115:138-45.
5. Pui CH. Acute lymphoblastic leukemia: Introduction. Semin Hematol 2009;46:1-2.
6. Hui RC, Francis RE, Guest SK, Costa JR, Gomes AR, Myatt SS, *et al.* Doxorubicin activates *FOXO3a* to induce the expression of multidrug resistance gene ABCB1 (MDR1) in K562 leukemic cells. Mol Cancer Ther 2008;7:670-8.
7. Martini M, De Santis MC, Braccini L, Gulluni F, Hirsch E. PI3K/AKT signaling pathway and cancer: An updated review. Ann Med 2014;46:372-83.
8. Tsai WB, Chung YM, Takahashi Y, Xu Z, Hu MC. Functional interaction between *FOXO3a* and ATM regulates DNA damage response. Nat Cell Biol 2008;10:460-7.
9. Ferber EC, Peck B, Delpuech O, Bell GP, East P, Schulze A, *et al. FOXO3a* regulates reactive oxygen metabolism by inhibiting mitochondrial gene expression. Cell Death Differ 2012;19:968-79.
10. Schmidt M, Fernandez de Mattos S, van der Horst A, Klompmaker R, Kops GJ, Lam EW, *et al.* Cell cycle inhibition by FoxO forkhead transcription factors involves downregulation of cyclin D. Mol Cell Biol 2002;22:7842-52.
11. Yang JY, Xia W, Hu MC. Ionizing radiation activates expression of *FOXO3a*, fas ligand, and bim, and induces cell apoptosis. Int J Oncol 2006;29:643-8.
12. Tran H, Brunet A, Grenier JM, Datta SR, Fornace AJ Jr., DiStefano PS, *et al.* DNA repair pathway stimulated by the forkhead transcription factor *FOXO3a* through the gadd45 protein. Science 2002;296:530-4.
13. Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, *et al.* FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell 2007;128:325-39.
14. Greer EL, Brunet A. FOXO transcription factors at the interface between longevity and tumor suppression. Oncogene 2005;24:7410-25.
15. Ausserlechner MJ, Salvador C, Deutschmann A, Bodner M, Viola G, Bortolozzi R, *et al.* Therapy-resistant acute lymphoblastic leukemia (ALL) cells inactivate FOXO3 to escape apoptosis induction by TRAIL and Noxa. Oncotarget 2013;4:995-1007.
16. Komatsu N, Watanabe T, Uchida M, Mori M, Kirito K, Kikuchi S, *et al.* A member of forkhead transcription factor FKHRL1 is a downstream effector of STI571-induced cell cycle arrest in BCR-ABL-expressing cells. J Biol Chem 2003;278:6411-9.
17. Kerdiles YM, Beisner DR, Tinoco R, Dejean AS, Castrillon DH, DePinho RA, *et al.* Foxo1 links homing and survival of naive T cells by regulating L-selectin, CCR7 and interleukin 7 receptor. Nat Immunol 2009;10:176-84.
18. Wang W, Lv L, Pan K, Zhang Y, Zhao JJ, Chen JG, *et al.* Reduced expression of transcription factor AP-2α is associated with gastric adenocarcinoma prognosis. PLoS One 2011;6:e24897.
19. Prasad SB, Yadav SS, Das M, Govardhan HB, Pandey LK, Singh S, *et al.* Down regulation of FOXO1 promotes cell proliferation in cervical cancer. J Cancer 2014;5:655-62.
20. Webb AE, Kundaje A, Brunet A. Characterization of the direct targets of FOXO transcription factors throughout evolution. Aging Cell 2016;15:673-85.
21. Polak R, Buitenhuis M. The PI3K/PKB signaling module as key regulator of hematopoiesis: Implications for therapeutic strategies in leukemia. Blood 2012;119:911-23.
22. Miyamoto K, Araki KY, Naka K, Arai F, Takubo K, Yamazaki S, *et al. Foxo3a* is essential for maintenance of the hematopoietic stem cell pool. Cell Stem Cell 2007;1:101-12.
23. Engström M, Karlsson R, Jönsson JI. Inactivation of the forkhead transcription factor foxO3 is essential for PKB-mediated survival of hematopoietic progenitor cells by kit ligand. Exp Hematol 2003;31:316-23.
24. Kubota Y, Ohnishi H, Kitanaka A, Ishida T, Tanaka T. Constitutive activation of PI3K is involved in the spontaneous proliferation of primary acute myeloid leukemia cells: Direct evidence of PI3K activation. Leukemia 2004;18:1438-40.
25. Chapuis N, Park S, Leotoing L, Tamburini J, Verdier F, Bardet V, *et al.* IκB kinase overcomes PI3K/Akt and ERK/MAPK to control *FOXO3a* activity in acute myeloid leukemia. Blood 2010;116:4240-50.
26. Ticchioni M, Essafi M, Jeandel PY, Davi F, Cassuto JP, Deckert M, *et al.* Homeostatic chemokines increase survival of B-chronic lymphocytic leukemia cells through inactivation of transcription factor *FOXO3a*. Oncogene 2007;26:7081-91.
27. Arimoto-Ishida E, Ohmichi M, Mabuchi S, Takahashi T, Ohshima C, Hayakawa J, *et al.* Inhibition of phosphorylation of a forkhead transcription factor sensitizes human ovarian cancer cells to cisplatin. Endocrinology 2004;145:2014-22.
28. Kikuno N, Shiina H, Urakami S, Kawamoto K, Hirata H, Tanaka Y, *et al.* Knockdown of astrocyte-elevated gene-1 inhibits prostate cancer progression through upregulation of *FOXO3a* activity. Oncogene 2007;26:7647-55.
29. Lin H, Dai T, Xiong H, Zhao X, Chen X, Yu C, *et al.* Unregulated miR-96 induces cell proliferation in human breast cancer by downregulating transcriptional factor *FOXO3a*. PLoS One 2010;5:e15797.
30. Yang XB, Zhao JJ, Huang CY, Wang QJ, Pan K, Wang DD, *et al.* Decreased expression of the *FOXO3a* gene is associated with poor prognosis in primary gastric adenocarcinoma patients. PLoS One 2013;8:e78158.
31. Gomes AM, Soares MV, Ribeiro P, Caldas J, Póvoa V, Martins LR, *et al.* Adult B-cell acute lymphoblastic leukemia cells display decreased PTEN activity and constitutive hyperactivation of PI3K/Akt pathway despite high PTEN protein levels. Haematologica 2014;99:1062-8.
32. Dewar R, Chen ST, Yeckes-Rodin H, Miller K, Khosravi-Far R. Bortezomib treatment causes remission in a Ph+ALL patient and reveals FoxO as a theranostic marker. Cancer Biol Ther 2011;11:552-8.
33. Tang YL, Huang LB, Lin WH, Wang LN, Tian Y, Shi D, *et al.* Butein inhibits cell proliferation and induces cell cycle arrest in acute lymphoblastic leukemia via *FOXO3a*/p27kip1 pathway. Oncotarget 2016;7:18651-64.