Expression of FOXP1 and p53 in Reactive Lymphoid Lesion and B-cell Non-Hodgkin Lymphoma, Large Cell Type

Ita Ellyana[1,2,3], Nila Kurniasari[4,5,6]

[1]Resident, Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, [2]Resident, Universitas Airlangga Hospital, Universitas Airlangga, Surabaya, Indonesia, [3]Resident, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, [4]Lecturer, Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia, [5]Lecturer, Universitas Airlangga Hospital, Universitas Airlangga, Surabaya, Indonesia, [6]Lecturer, Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Abstract

Lymphoproliferative lesions that have morphology between benign and malignant are difficult to diagnose even with immunohistochemical and clonality testing. The correct diagnosis is necessary for the prompt treatment. These lesions can also serve as instructive models of lymphomagenesis. FOXP1 plays an important role in B-cell development, has a potential oncogene in B-cell Non-Hodgkin lymphoma, and p53 protein has a crucial role in the regulation of cell cycle, DNA repair, apoptosis, and senescence tumor suppression activity. In this study, we analyze the role of FOXP1 and p53 expression in reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type. 68 paraffin blocks samples from patients diagnosed as reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type was sectioned and stained with immunohistochemistry for FOXP1 and p53, and the percentage of nuclear cells showing positive staining were evaluated. Expression of FOXP1 and p53 in B-cell Non-Hodgkin lymphoma, large cell type is higher than in reactive lymphoid hyperplasia with p=0.001 and cutoff point 45%(CI=95%) for FOXP1 and p=0.001 and cutoff point 7.5%(CI=95%) for p53. There is a significant correlation between the expression of FOXP1 and p53 in reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type (p=0.001). Our findings suggest that high expression of FOXP1 and p53 in B-cell Non-Hodgkin lymphoma may demonstrate the role of FOXP1 and p53 in lymphomagenesis and these markers may help to distinguish benign and malignant lymphoproliferative lesions.

Keywords: *reactive lymphoid hyperplasia, B-cell Non-Hodgkin lymphoma, FOXP1, p53*

Introduction

Lymph nodes are major components in the immune system. They react to various stimuli by undergoing reactive changes[1]. Recent research identifies lymphoproliferative lesions that interface between benign lesions and malignancies[2]. Distinguishing the reactive lymph node from a neoplastic lymphoproliferative process is one of the very crucial things in order to avoid harmful treatment for patients who do not need the therapy and vice versa[3]. Detection of clonality in a suspected lymphoproliferative lesion is important in diagnostic criteria[4], such as Bcl2/IGH and CCND1/IGH translocation associated with in situ forms of follicular lymphoma and mantle cell lymphoma, but sometimes clonal populations of B and T lymphocytes have been identified in many reactive or infectious disorders, and many lymphoma- or leukemia-associated translocations have been identified in the peripheral blood of healthy individuals[2].

The aim of this research is to study the role FOXP1 and p53 in lymphomagenesis, so this may help to distinguish borderline lesions between atypical

Corresponding author:
Nila Kurniasari
Email: nila-k@fk.unair.ac.id

lymphoproliferative lesions and malignant lymphoma. FOXP1 has an important role in regulation of B-cell development and maturation[5], also known as an oncogene in various types of B-cell Non-Hodgkin lymphoma[6], while p53 is the most important molecular marker in malignancy including diffuse large B-cell lymphoma. Loss of normal p53 activity is associated with lymphomagenesis and mediates tumors resistant to chemotherapy[7]. In this study, the truly benign lesions (reactive lymphoid hyperplasia) and truly malignant lesions (B-cell Non-Hodgkin lymphoma, large cell type) were used to represent the benign lymphoproliferative lesions and malignant lymphoproliferative lesions.

Materials and Methods

This study had been approved by the Health Research Ethic Committee of Dr. Soetomo General Hospital, Surabaya, Indonesia (0040/LOE/301.4.2/VI/2020).

Research Design and Sample

This was an analytic observational research with a cross-sectional approach. There were 68 samples, each of 34 formalin-fixed, paraffin-embedded tissues were obtained from patients diagnosed as reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type patients during 2017-2018 in Anatomical Pathology Laboratory, Dr. Soetomo General Hospital, Surabaya, Indonesia.

Immunohistochemical Staining

The formalin-fixed, paraffin-embedded tissues were cut into 4 mm sections, deparaffinized with xylol for 5 minutes three times, and rehydrated through graded alcohol. Antigen retrieval was achieved by microwave treatment in sodium citrate buffer (pH 6.0) for 10 minutes. The tissue sections were then incubated with monoclonal antibodies for FOXP1(CMC35032010; dilution 1:400; Cell Marque, Abcam Technology) and p53(DS-0337-C; dilution 1:100; Diagnostic Bio System) overnight, followed by secondary antibody for 10 minutes at room temperature. Sections were then counterstained with hematoxylin and dehydrated with alcohol.

Evaluation of Immunohistochemical Expression

All samples were evaluated blindly by 2 observers. This study assessed the expression of FOXP1 and p53 by calculating the percentage in the entire field of view using a light microscope, Olympus (40× magnification) [8,9]. The percentage of FOXP1 and p53 in the entire field of view was calculated using a semiquantitative method by dividing the number of mature lymphocyte cells in reactive lymphoid hyperplasia and tumor cells in B-cell Non-Hodgkin lymphoma, large cell type that are stained brown in the nuclei by the total population of the sample cells.

Statistical Analysis

All statistical analyses were calculated using SPSS v25.0. The comparison of FOXP1 expression in reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell types was tested using unpaired T test. The comparison of p53 expression in reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type was tested using Mann Whitney U test. The correlation was analyzed using Spearman test, with a significance level <0.05 ($p < 0.05$).

Results and Discussion

The clinicopathological characteristics of the patients are shown in Table 1. FOXP1 was expressed at the nuclei of mature lymphocytes in the reactive hyperplasia and nuclei of tumor cells in Non-Hodgkin lymphoma (Figure 1). A significant difference of FOXP1 expression was found between reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type ($p = 0.001$) (Table 2), which was higher in B-cell Non-Hodgkin Lymphoma, large cell type than in reactive lymphoid hyperplasia, with cutoff point 45%(CI=95%). Pearson correlation test showed a significant correlation between FOXP1 expression of reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type ($r_p = 0.793$, $p = 0.001$). The expression of p53 was expressed at the nuclei of mature lymphocytes in the reactive hyperplasia and nuclei of tumor cells in Non-Hodgkin lymphoma (Figure 2). A significant difference of p53 expression was found ($p = 0.001$) (Table 3) and expression in B-cell Non-Hodgkin lymphoma, large cell type is higher than in reactive lymphoid hyperplasia with cutoff point 7.5%(CI=95%). Spearman correlation test showed a significant correlation between p53 expression in reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type ($r_s = 0.768$, p =

0,001). The expression of FOXP1 and p53 in reactive lymphoid hyperplasia and B-cell Non-Hodgkin Lymphoma, large cell type has a significant positive correlation (r_s = 0.640, p = 0,001) and shown in Table 4.

Table 1. Clinicopathological characteristics of the patients.

Characteristics		
Age (years)	**Reactive Lesion (n) (%)**	**B-cell NHL (n) (%)**
0-10	9 (26.47%)	0
11-20	1 (2.94%)	0
21-30	3 (8.82%)	2 (5.88%)
31-40	3 (8.82%)	3 (8.82%)
41-50	6 (17.64%)	4 (11.76%)
51-60	8 (23.53%)	14 (41.17%)
61-70	4 (11.76%)	8 (23.53%)
71-80	0	2 (5.88%)
>80	0	1 (2.94%)
Mean age	35.5±23.36	53.73±13.60
Age range	2-70	25-81
Sex	(n (%))	(n (%))
Male	26 (76.5%)	22 (64.7%)
Female	8 (23.5%)	12 (35.3%)
Location	(n (%))	(n (%))
Neck	16 (47.06%)	16 (47.06%)
Mesenterium	6 (17.64%)	0
Axilla	2 (5.88%)	0
Submandibule	4 (11.76%)	0
Tonsil	1 (2.94%)	4 (11.76%)
Intraabdomen	2 (5.88%)	3 (8.82%)
Inguinal	3 (8.82%)	2 (5.88%)
Extranodal	0	9 (26.47%)

Table 2. Expression of FOXP1 in Reactive Lymphoid Hyperplasia and B-Cell Non-Hodgkin Lymphoma.

Status	Mean	SD	Min	Max	p value*
Reactive Lesion B-cell NHL	44.4 83.6	15.99 14.59	5 38	88 90	0.001

#Unpaired T test applied

p-value <0.05, considered as significant

Table 3. Expression of p53 in Reactive Lymphoid Hyperplasia and B-Cell Non-Hodgkin Lymphoma.

Status	Mean	SD	Min	Max	*P* value*
Reactive Lesion B-cell NHL	10.61 55.38	30.72 30.72	0 5	30 98	0.001

#Mann Whitney U test applied

p-value < 0.05, considered as significant

Table 4. Correlation between FOXP1 and p53 expression in Reactive Lymphoid Hyperplasia and B-Cell Non-Hodgkin Lymphoma.

		FOXP1 expression
p53 expression	r_s	0.640
	p-value n	0.001 68

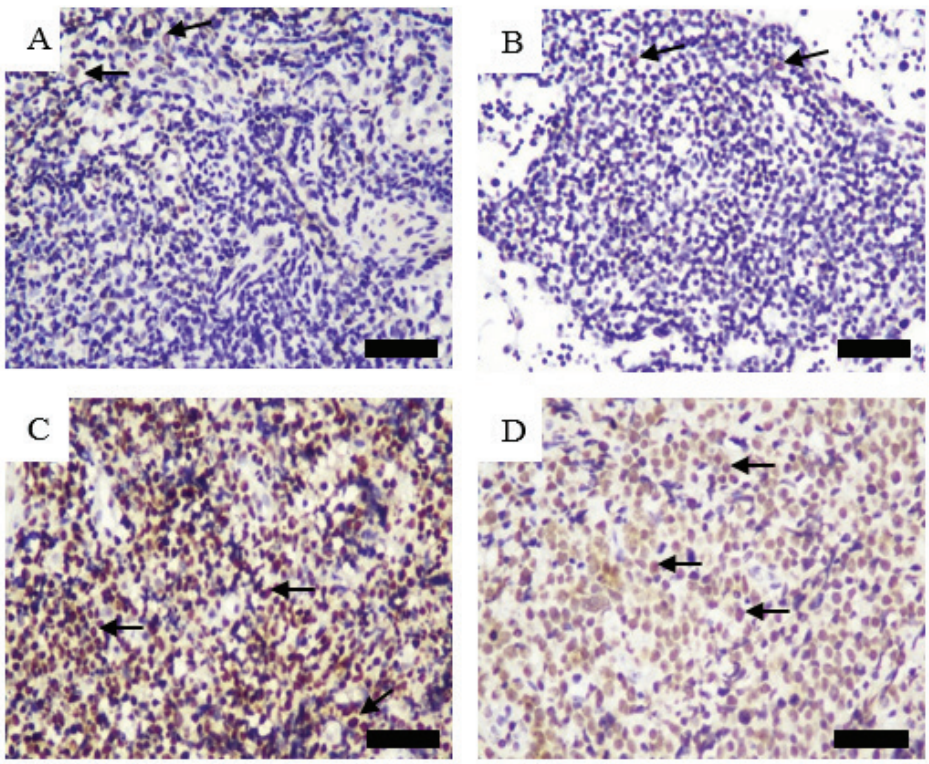

Figure 1. Immunohistochemical expression of FOXP1(brown staining in nuclei) (400× magnification). A,B. Immunohistochemical expression of FOXP1 in reactive lymphoid hyperplasia (A: 30% staining, B: 60% staining). C,D. Immunohistochemical expression of FOXP1 in B-cell Non Hodgkin Lymphoma, large cell type (C: 90% staining, D: 98% staining). Black arrows: FOXP1 expression; bar: 50 µm.

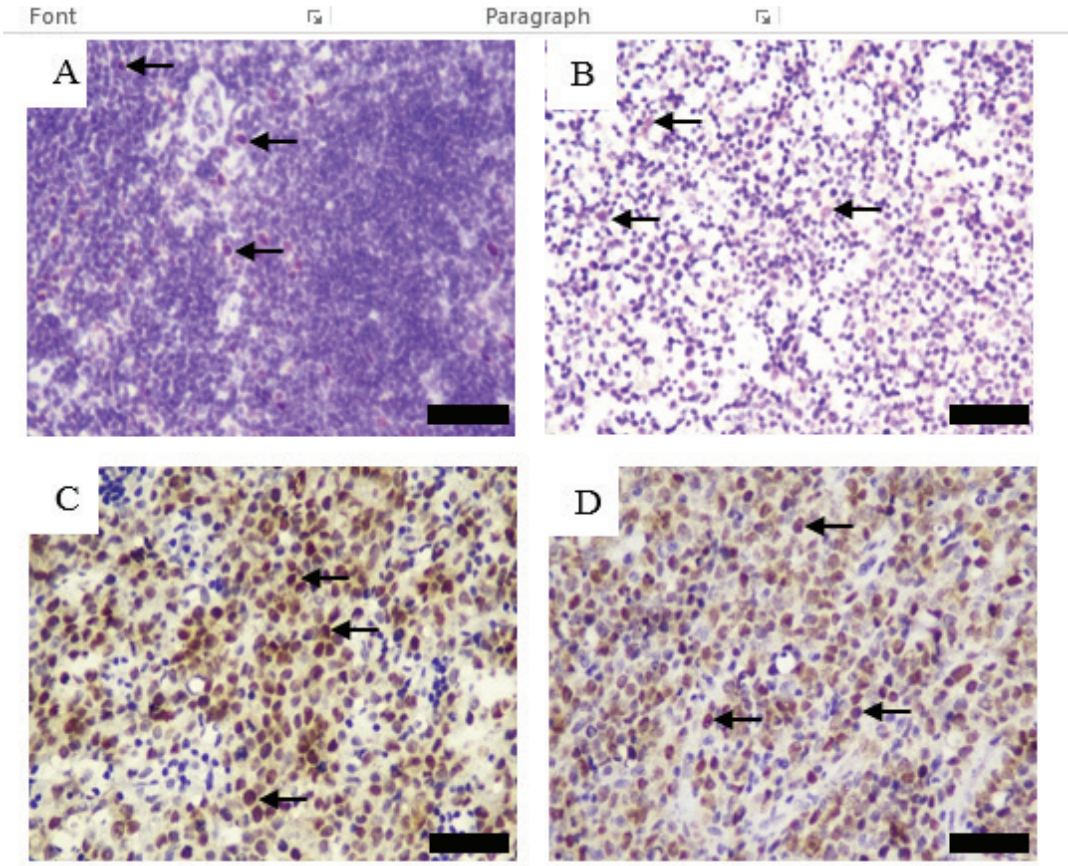

Figure 2. Immunohistochemical expression of p53 (brown staining in nuclei) (400× magnification). A,B. Immunohistochemical expression of p53 in reactive lymphoid hyperplasia (A: 20% staining, B:30% staining). C,D. Immunohistochemical expression of p53 in B-cell Non Hodgkin Lymphoma, large cell type (C: 90% staining, D: 98% staining). Black arrows: p53 expression; bar: 50 µm.

Lymphoproliferative lesions with similar morphologic feature could be a difficult area in histopathology. Therefore, detection of clonality in these cases plays important role to diagnostic criteria. The key characteristic of cancer is the monoclonality of tumor cells, which derivatives of transformed malignant cells, which should be done in the dubious conditions[4]. Ideally, further immunohistochemical examination also needed to determine the subtypes of Non-Hodgkin lymphoma with similar morphology [10], for example, the subtypes of DLBCL are determined by gene expression profiling, which is the gold standard for identifying the GCB and ABC subtypes, but is not routine practice[3].

Our study showed that FOXP1 expression was higher in B-cell Non-Hodgkin lymphoma, large cell type than reactive lymphoid hyperplasia and both of them showed significant correlation. Patzelt et al stated that FOXP1 transcription factors are important in the early development of B-cell. FOXP1 is deregulated through chromosome translocation in mature B-cell lymphoma, including diffuse large B-cell lymphoma (DLBCL). Deficiency of FOXP1 in early lymphoid precursors results in the cessation of pro-B cell transitions to pre-B and reduces peripheral matured B-cell. Overexpression of FOXP1 in DLBCL and B-cell suppresses several proapoptotic genes such as Bik, Eaf2, and Hrk, in collaboration with NF-κB activity to support B-cell resistance. It is suspected that high expression of FOXP1 in lymphoma cells prevents cells from undergoing apoptosis. FOXP1 knockdown on DLBCL cells induces an increase in MHC II expression, so that it can have an effect on immunosurveillance tumors during lymphomagenesis[11].

Expression of p53 also showed higher in B-cell Non-Hodgkin lymphoma, large cell type than reactive lymphoid hyperplasia and both of them are significantly correlated. This result is similar with research by Kanavaros *et al*. who found that in non-neoplasmic conditions, the role of p53 besides inhibiting the cell cycle is also involved in apoptosis. The p53 protein influences the expression of Bcl2 and Bax, which are involved in the regulation of apoptosis. Bcl2 which acts as an antiapoptotic undergoes downregulation, whereas Bax which induces apoptosis experiences upregulation by p53. The proapoptotic effect of Bax arises through performing activity as opposed to Bcl2. Bax also works as a tumor suppressor gene. Several studies have shown that Bcl2 and Bax proteins play a role in lymphoid malignancies[12]. Genetic factors that disrupt DNA repair or apoptosis can increase precancerous risk[13].

The wild type p53 is tightly controlled at the post translational stage. Under physiological conditions, the level of wild p53 is low due to constitutive degradation by E3 ubiquitin ligase MDM2 which is a target of wild type p53 transcription. Conversely tumors that have mutant p53 are typically characterized by a substantial accumulation of p53 protein[14]. The p53 gene mutation and dysregulation of the p53 pathway are important in the pathogenesis of cancers including lymphomas. TP53 dysfunction in lymphoid malignancies can occur at the level of DNA, mRNA, or protein in cis or trans. Many mutant p53 work in a dominant-negative way to inhibit the function of wild-type p53. Single allele mutations are often followed by loss of heterozygosity, which then supports tumor development. The p53 mutation points in lymphoma malignancies occur most often in the p53 DNA-binding domain (DBD). Simultaneously, p53 and inactivation of other genes, for example p21, cause a poor prognostic effect. The combination of p53+/p21- immunophenotype can reflect the p53 mutation with prognostic value[15].

We found that FOXP1 and p53 expression in reactive lymphoid hyperplasia and B-cell Non-Hodgkin lymphoma, large cell type was significantly correlated. Study by He *et al*., described the activity of FOXP1 depends on miR34α downregulation, which allows the development of B-cell. Inactivation of miR34α influences the pathway of B-cell development, consistent with the abnormality seen with the expression of FOXP1, p53, and Bcl2 that affect the development of mature B-cell and supports malignant transformation associated with B-cell lymphoma[16]. Networks of p53 through FOXP1 and Bcl2 are connected by miR34α which is a tumor suppressor. The effect of miR34α on FOXP1 which suppresses p53 is potentially oncogenic on post germinal center B-cell. Parallel effects also occur on the role of miR34α as a link between p53 and the oncogenic protein Bcl-2. The effect of p53 on the B-cell developmental pathway is consistent with the abnormalities found in p53 deficiency, namely the increasing number of pre-B cell as well as B-cell, which is also a consequence of the loss of miR34α function[17]. The effect of miR34α on FOXP1 in the form of the mechanism of p53 suppressing tumor cells implies a connection between p53 and FOXP1 through the action of miR34α[16].

Conclusion

In summary, the expression of FOXP1 and p53 in B-cell Non-Hodgkin lymphoma, large cell type, is higher than in reactive lymphoid hyperplasia. This suggest that FOXP1 and p53 have a role in lymphomagenesis and these markers may help to distinguish benign and malignant lymphoproliferative lesions. The suspicion for lymphoid malignancy will be raised if the expression of FOXP1 is above 45% and p53 is above 7.5%. Further investigation in other types of lymphoma such as low-grade lymphoma should be carried out because they have different biology behavior

Conflict of Interest: The authors declare that they have no conflict of interest.

Source of Funding : This study supported by the Ministry of Education and Culture of the Republic of Indonesia.

Acknowledgements : We thank Arif Nur Muhammad Ansori for editing the manuscript.

Ethical Approval: This study had been approved by the Health Research Ethic Committee of Dr. Soetomo General Hospital, Surabaya, Indonesia (0040/LOE/301.4.2/VI/2020).

References

1. Rosai, J. Rosai and Ackerman's Surgical Pathology. Tenth Edition. London: Elsevier Inc. p. 1771-1780;

2011.

2. Ganapathi KA, Pittaluga S, Odejide OO, Freedman AS, Jaffe ES. Early lymphoid lesions: conceptual, diagnostic and clinical challenges. Haematologica. 2014; 99(9): 1421-32.
3. Slack GW. The pathology of reactive lymphadenopathies: A discussion of common reactive patterns and their malignant mimics. Arch Pathol Lab Med. 2016; 140(9): 881–92.
4. van Krieken JHJM, Langerak AW, Macintyre EA, Kneba M, Hodges E, Sanz RG, et al. Improved reliability of lymphoma diagnostics via PCR-based clonality testing: - Report of the BIOMED-2 Concerted Action BHM4-CT98-3936. Leukemia. 2007; 21(2): 201–6.
5. Rouhigharabaei L, Ferreiro JF, Tousseyn T, Van Der Krogt JA, Put N, Haralambieva E, et al. Non-IG aberrations of FOXP1 in B-cell malignancies lead to an aberrant expression of N-truncated isoforms of FOXP1. PLoS One. 2014; 9(1).
6. Yu BH, Zhou XY, Li BZ, Xiao XY, Yan SY, Shi DR. FOXP1 expression and its clinicopathologic significance in nodal and extranodal diffuse large B-cell lymphoma. Ann Hematol. 2011; 90(6): 701–8.
7. Wang XJ, Medeiros LJ, Bueso-Ramos CE, Tang G, Wang S, Oki Y, et al. P53 expression correlates with poorer survival and augments the negative prognostic effect of MYC rearrangement, expression or concurrent MYC/BCL2 expression in diffuse large B-cell lymphoma. Mod Pathol. 2017; 30(2): 194–203.
8. Xie Y, Ajaz Bulbul M, Ji L, Inouye CM, Groshen SG, Tulpule A, et al. P53 expression is a strong marker of inferior survival in de novo diffuse large B-cell lymphoma and may have enhanced negative effect with MYC coexpression: A Single Institutional Clinicopathologic Study. Am J Clin Pathol. 2014; 141(4): 593–604.
9. Banham AH, Connors JM, Brown PJ, Cordell JL, Ott G, Sreenivasan G, et al. Expression of the FOXP1 transcription factor is strongly associated with inferior survival in patients with diffuse large B-cell lymphoma. Clin Cancer Res. 2005; 11(3): 1065–72.
10. Montgomery ND, Fedoriw Y. Pathology consultation on intermediate-to-large B-cell lymphomas. Am J Clin Pathol. 2014; 141(3): 305–17.
11. Patzelt T, Keppler SJ, Gorka O, et al. Foxp1 controls mature B cell survival and the development of follicular and B-1 B cells. Proc Natl Acad Sci U S A. 2018; 115(12): 3120-3125.
12. Kanavaros P, Stefanaki K, Vlachonikolis J, et al. Expression of p53, p21/waf1, bcl-2, bax, Rb and Ki67 proteins in Hodgkin's lymphomas. Histol Histopathol. 2000; 15(2): 445-453.
13. Voropaeva EN, Pospelova TI, Voevoda MI, Maksimov VN, Orlov YL, Seregina OB. Clinical aspects of TP53 gene inactivation in diffuse large B-cell lymphoma. BMC Med Genomics. 2019; 12(Suppl 2): 35.
14. Jethwa A, Słabicki M, Hüllein J, Jentzsch M, Dalal V, Rabe S, et al. TRRAP is essential for regulating the accumulation of mutant and wild-type p53 in lymphoma. Blood. 2018; 131(25): 2789–802.
15. Xu-Monette ZY, Medeiros LJ, Li Y, Orlowski RZ, Andreeff M, Bueso-Ramos CE, Greiner TC, McDonnell TJ, Young KH. Dysfunction of the TP53 tumor suppressor gene in lymphoid malignancies. Blood. 2012; 119(16): 3668-83.
16. He M, Gao L, Zhang S, Tao L, Wang J, Yang J, et al. Prognostic significance of miR-34a and its target proteins of FOXP1, p53, and BCL2 in gastric MALT lymphoma and DLBCL. Gastric Cancer. 2014;17(3):431–41.
17. Rao DS, O'Connell RM, Chaudhuri AA, Garcia-Flores Y, Geiger TL, Baltimore D. MicroRNA-34a perturbs B lymphocyte development by repressing the forkhead box transcription factor Foxp1. Immunity. 2010; 33(1): 48-59.