Original article

Expression of Blimp-1 transcription factor mRNA isoforms in the mononuclear cells from bone marrow of leukemia and lymphoma patients

Jedsada Kaewrakmuk,[1] Laddawan Laomanit,[1] Mongkol Punyot,[1]
Pimlak Charoenkwan,[2] Weerasak Nawarawong,[3] and Wilaiwan Petsophonsakul[1]
[1]Department of Microbiology, [2]Department of Pediatrics, [3]Department of Internal Medicine, Faculty of Medicine, Chiang Mai University

Objective To determine the expression of Blimp-1 mRNA isoforms in the mononuclear cells from bone marrow of patients with leukemia and lymphoma.

Method Twenty three bone marrow samples were obtained from leukemia or lymphoma patients at Maharaj Nakorn Chiang Mai University Hospital. Bone marrow mononuclear cells were isolated by Ficoll-Hypaque gradient centrifugation. The expression of full length and exon 7 deleted (△7 isoform) mRNA was performed by RT-PCR. The Molecular Analysis program was used to analyze intensity of the PCR products. The band intensity ratio of Blimp-1 isoforms was compared with the GAPDH control of the same sample.

Results Blimp-1 mRNA isoforms were found to have abnormal expressions in some patients. Most patients who expressed both isoforms presented more △7 isoforms than full length ones, whereas of four patients; two with ALL, one with AML and one with lymphoma, were found to have the opposite ratio. Only △7 isoform was expressed in 2 patients with NHL, who were both new cases of untreated patients. Interestingly, one abnormal isoform with an unusual PCR product size of 1,150 bp was found in a patient with ALL. This patient also showed an abnormal expression of XBP-1, a downstream signaling of Blimp-1 that responds to ER stress and is involved in immunoglobulin secretion.

Conclusion This study demonstrated the expression profile of full-length and △7 Blimp-1 isoforms in primary leukemic and lymphoma cells. One isoform, which has not been described, was identified in one patient diagnosed with ALL. As △7 isoforms lack a DNA binding domain, an abnormally high level of △7 isoform expression may play a role in the malignant transformation of these cells. **Chiang Mai Medical Journal 2016;55(Suppl 1): 67-74.**

Keywords: Blimp-1 isoform, exon7 deleted isoform, XBP-1, bone marrow, leukemia, lymphoma patient

Address correspondence to: Wilaiwan Petsophonsakul, Dr.rer.nat., Department of Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand. E-mail: wilaiwan.pet@cmu.ac.th

Received: May 6, 2015; **Accepted**: July 22 2015.

Introduction

Blimp-1 is a transcriptional repressor that has been recognized as a master regulator of terminal B cell differentiation. Expression of Blimp-1 is sufficient for driving B cells to differentiate into plasma cells. Blimp-1 regulates gene expression programs including cell cycles, immunoglobulin secretion, and genes required for GC or activated B cells[1,2]. It also has been found to regulate terminal differentiation of other cell lineages such as myeloid cells, epithelial cells and subsets of effector T cells[3,4]. Later, Blimp-1 was found to play a role in homeostasis and regulating self-tolerance[5].

Blimp-1 is a zinc finger-containing protein encoded by *prdm1*. In mouse, the five zinc finger domains critical for DNA binding are encoded in exon 6 and 7. This intron-exon structure for the zinc finger domains is conserved in human *prdm1*. Several Blimp-1 mRNA isoforms have been found in mouse plasmacytoma cells[6]. The 3 isoforms of Blimp-1 mRNA were generated by differential usage of polyadenylation sites, and a minor isoform resulted from differential splicing of exon 7 (Δ7 isoform). Exon 7 encodes part of zinc finger 1, all of zinc finger 2 and part of zinc finger 3. The Δ7 is expressed at less than 10% of the total Blimp-1 mRNA. Both the human and mouse Blimp-1 gene show a very similar exon-intron organization, except for the 5'UT in human, which is encoded in only one exon, while that in mouse is encoded in 2 exons. Thus, mouse exon 7 is comparable to exon 6 in human. In this study, the Δ7 isoform was used, as applied originally to mouse, instead of exon 6 for human.

Regulation of cell function by isoforms has been reported in other transcription factors such as XBP-1, Ikaros family[7,8]. During the early and terminal phase of B cell differentiation, several transcription factors such as PAX-5, c-myc, Bcl-6, IRF-4 as well as XBP-1 are coordinated and cross regulated with each other. Blimp-1 has been proposed as a master of regulation in the differentiation to plasma cells, and XBP-1 is a downstream transcription factor that is regulated by Blimp-1[1,2]. XBP-1 has 2 isoforms; spliced isoform which is active, whereas the unspliced one is not. Plasma cell differentiation is dependent on UPR (unfold protein response) induced IRE1α splicing of XBP-1[8]. XBP1 splicing mediates a major adaptive pathway and identifies cells undergoing an active stress response. Spliced XBP-1 expression delineates heterogeneity amongst plasma cell-derived neoplasms (multiple myeloma) and plasmablastic diffuse large B-cell lymphomas[9-11].

Blimp-1 isoform has demonstrated relevance as a PRDI-BF1β isoform in patients with multiple myeloma[12]. This isoform of human Blimp-1 is generated by transcription initiation at an alternative promoter located at 5 of exon 4 in human *PRDM1*[2]. The PRDI-BF1 deleted protein (called PRDI-BF1β) lacks 101 amino acids that comprise most of the regulatory domain. Since this molecule contains the DNA-binding domain, but bears a disrupted regulatory domain, PRDIBF1β might behave as an inhibitor of functional PRDIBF1. Interestingly, the transcription level for this isoform was markedly low in normal human plasma cells, but far higher in malignant ones, thus suggesting interference with the normal cell differentiation program.

Compared to PRDI-BF1β isoforms, Δ7 Blimp-1 and full length isoforms have been found to express in many cell types including non B cell lineage and normal cells. Thus, the Δ7 Blimp-1 does not result from an aberrant process, but is a normal isoform that has been expressed physiologically. Interestingly, steady state mRNA encoding Δ7 Blimp-1 showed an expression lower than that encoding full length protein. Thus, merely a larger amount of full length rather than Δ7 Blimp-1 isoform might not be sufficient to drive the function of Blimp-1, but a certain high level of full length is needed. It would be interesting to suggest that the Δ7 Blimp-1 isoform interferes with the function of full length Blimp-1. Since the Δ7 Blimp-1 isoform is unable to bind DNA, it would likely interfere with full length Blimp-1 and render it also unable to bind DNA.

This study compared the levels of full length and Δ7 isoforms in primary leukemic and lymphoma cells from bone marrow of leukemia and lymphoma patients. It speculated that the Δ7 isoform level of expression may be abnormal in hematological malignancies in some patients, and that might lead to uncontrolled cell division and normal function of immune cells.

Method

1. Patient samples and cell preparation

Twenty three bone marrow samples were obtained from leukemia or lymphoma patients, who were treated at Maharaj Nakorn Chiang Mai Hospital. They were collected from existing/disposed specimens for therapeutic propose, involving no more than minimal risk and this study was approved by the ethical committee of the Faculty of Medicine, Chiang Mai University, Thailand. Mono nuclear cells were isolated by Ficoll-Hypaque gradient.

2. cDNA preparation and RT-PCR

RNAs from bone marrow mononuclear cells were isolated using the RNeasy column (QIAGEN, GmbH Germany). The reverse transcription was performed using the RevertAID™ First Strand cDNA synthesis kit (Fermentas). The cDNA were amplified for Blimp-1 and GAPDH using the PCR Master Mix kit (Fermentas). The PCR conditions for each cycle were as follows: 94 °C for 30 sec, 50 °C for 30 sec, and 72 °C for 30 sec x 30 cycles. The final extension phase was performed at 72 °C for 10 min. The nested-primer PCR for Blimp-1 was performed under the same parameters for an additional 30 cycles.

3. Primer design

The sequences of the primers were designed and used to amplify Blimp-1 isoforms, the GAPDH housekeeping gene (control) and XBP-1 as shown in the Diagram 1 and Table 1 below:

4. Analysis of gene expressions

The Blimp-1 PCR products were run on 1.5% agarose gel containing ethidium bromide in a TBE buffer. The Molecular Analysis version 1.4

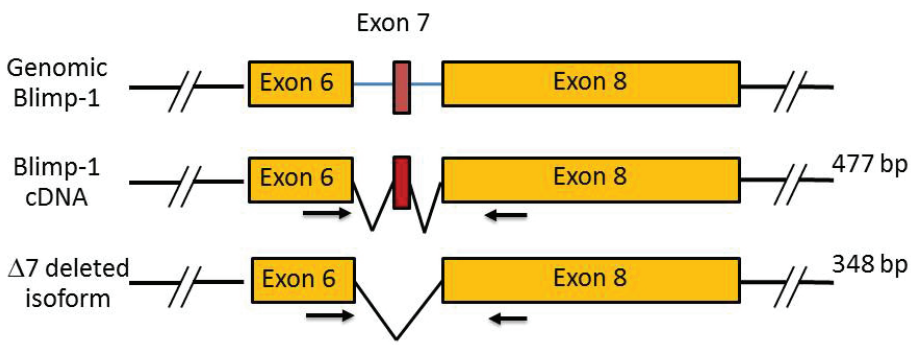

Diagram 1. Blimp-1 isoform primer mapping. Sense and anti-sense primers were designed to bind between exon 6 and exon 8 on the Blimp-1 cDNA, which provided a PCR product of 477 bp for a full length isoform and a smaller 348 bp for a Δ7 isoform

Table 1. Primer sequences of the Blimp-1 isoforms, GAPDH and XBP-1 used for PCR.

Gene	Sense	Antisense	PCR product (bp)	
			Full length	Δ7
Blimp-1	5'-GAC GAA GCC ATG AAT CTC A-3'	5'-TGA GGC TAC AGA GAT GGA T -3'	477	348
Nested Blimp-1	5'-CGA AGC CAT GAA TCT CAT TAA AAA-3'	5'-GCT ACA GAG AT G GAT GTA GTT C -3'	475	344
GAPDH	5'-GGT CAT CCC TGA GCT GAA CG-3'	5'-TCG TTG TCA TAC CAG GAA AT-3'	295	
XBP-1	5'-TAC ACT GCC TGG AGG ATA GC-3'	5'-GTT CCC GTT GCT TAC AGA AG-3'	339	

program was used to analyze intensity of the PCR products. The intensity ratio of full length and Δ7 Blimp-1 isoforms was compared between the patients.

Results

1. Expression of exon 7 deleted Blimp-1 isoform in normal human peripheral blood mononuclear cells

As Δ7 isoforms have been found in the mouse plasmacytoma cell line (P3X), an initial step was to determine whether they express in human in a similar pattern. Blimp-1 also expressed in several cell types besides plasma cells, therefore, an expression profile of Δ7 isoform was monitored in normal peripheral blood mononuclear cells as a control. The peripheral blood mononuclear cells showed an expression of both full length and Δ7 isoforms, as shown in Figure 1. PCR products were sequenced and they confirmed the expression in human, which was similar to that in mouse. The XBP-1, which is a downstream transcription factor regulated by Blimp-1, also was determined in order to investigate further if any aberrant expression of Blim-1 isoforms was found in any patients.

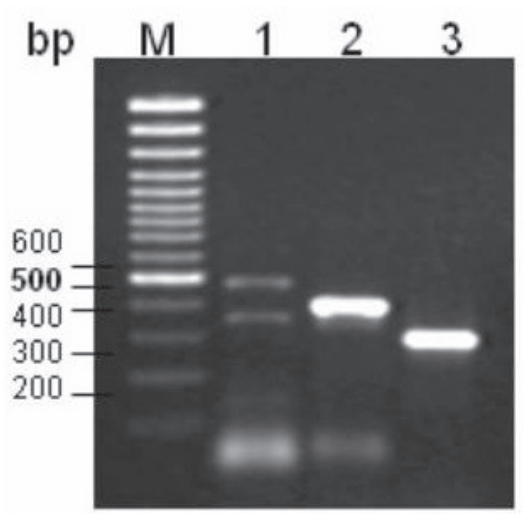

Figure 1. Expression of Blimp-1 isoform mRNA in normal peripheral blood mononuclear cells. Lane 1: Blimp-1 full length (475 bp) and Δ7 isoforms (344 bp), lane 2: XBP-1(399 bp), and lane 3: GAPDH control (295 bp)

2. Expression of the Blimp-1 Δ7 isoform in mononuclear cells from patient bone marrow

Both full length and delta7 Blimp-1 isoforms were found in most patients as shown in Figure 2. Most of the patients presented more Δ7 isoforms than full length ones, but 2 patients with ALL, one with relapsed AML (patient's code 12P and 13P) and one with lymphoma (patient's code 10V) expressed the opposite. Only Δ7 isoform was expressed in 2 patients with NHL (patient's code 2V and 5V), who were both new cases of untreated patients. The intensity of PCR products is represented by the ratio between full length and Δ7 isoforms. The intensity ratio of full length to Δ7 isoforms in all patient samples was between 0.08-2.30.

Interestingly, one abnormal isoform with an unusual PCR product size of 1,150 bp, and multiple fading products were found in a patient (25P) with ALL as shown in Figure 3. Blimp-1 is a master regulator of terminal B cell differentiation that regulates several downstream transcription factors, which might be affected by an abnormal expression of Blimp-1 isoforms. Thus, XBP-1, which is a downstream transcription factor, was monitored in this patient. The XBP-1 PCR product was barely visible, while the GAPDH control was normal. Other patients showed normal expression of XBP-1 (unpublished data). To investigate other transcription factors further and discover the origin of abnormality that leads to malignancy is of interest.

Discussion

In this study, Blimp-1 full length and Δ7 isoform expressions were studied in human bone marrow cells. The normal peripheral blood mononuclear cells showed an expression of both isoforms. PCR products were sequenced and they confirmed the expression in human, which was similar to that in mouse. The expression profile of both isoforms was then investigated in bone marrow mononuclear cells of patients with leukemia and lymphoma. As Blimp-1 expression was very low and limited by the amount of bone

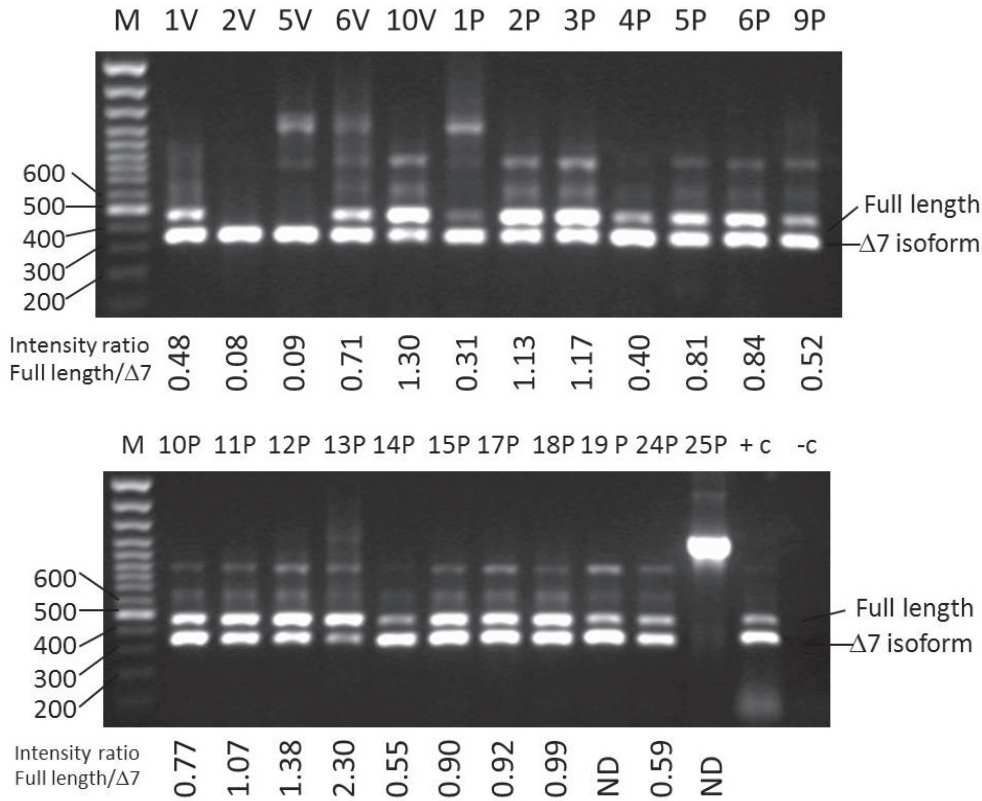

Figure 2. Expression of Blimp-1 isoform mRNA in mononuclear cells from patient bone marrow samples. Each lane of gel is represented in patient codes, a) marker, lane 1 (1V) CHL*, lane 2 (2V) NHL*, lane 3 (5V) NHL*, lane 4 (6V) NHL*, lane 5 (10V) myeloma*, lane 6 (1P) ALL, lane 7 (2P) ALL, lane 8 (3P) AML relapse*, lane 9 (4P) AML*, lane 10 (5P) ALL, lane 11 (6P) ALL* and lane 12 (9P) AML, b) marker, lane 1 (10P) ALL, lane 2 (11P) ALL, lane 3 (12P) ALL, lane 4 (13P) AML relapse, lane 5 (14P) ALL, lane 6 (15P) ALL, lane 7 (17P) ALL, lane 8 (18P) ALL, lane 9 (19P) ALL, lane 10 (24P) ALL, lane 11 ALL (25P), lane 12 positive control and lane 13 negative control.

Abbreviations: ALL: acute lymphoblastic leukemia, NHL: Non Hodgkin lymphoma, CHL: classical Hodgkin lymphoma. ND –not done, * New case

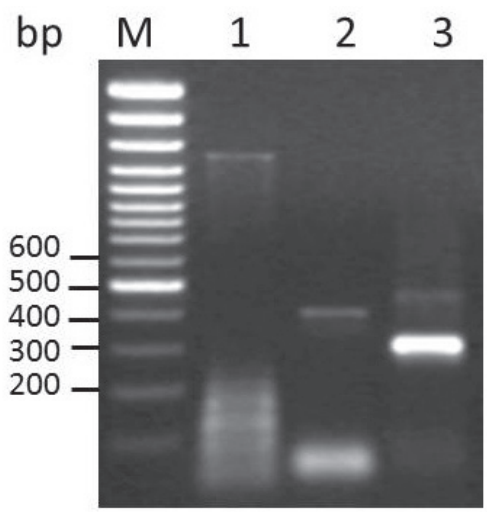

Figure 3. Expression of Blimp-1 isoform mRNA in mononuclear cells from a patient bone marrow sample. Lane M-100-bp ladder; lane 1-PCR product of Blimp-1 (1,150 bp); lane 2-PCR product of XBP-1 (399 bp); and lane 3 - PCR product GAPDH control (295 bp).

marrow sample, a nested PCR was used in order to increase sensitivity, and semi quantitative PCR was determined as a general expression profile. Accordingly, only remarkable abnormal expression was discussed in this study. Hybridization of PCR products for each sample must be determined quantitatively in order to give a precise value.

Due to ethical difficulty, the Blimp-1 isoform expression pattern was compared among this group of patients. Some patients were diagnosed normal bone marrow, which might be assumed as normal expressions or controls. Most of them presented more Δ7 isoforms than full length ones, whereas, 2 patients with ALL, one with AML and one with lymphoma expressed the opposite. Interestingly, two patients with NHL expressed only Δ7 isoform. Furthermore, one abnormal isoform with an unusual PCR product size of 1,150 bp, and multiple fading products, were found in a patient with ALL. This patient also showed an abnormal expression of XBP-1, which is a transcription factor that is regulated by Blimp-1. This will be investigated further in order to determine whether these abnormal isoforms result in malignant transformation.

Bone marrow cells consist of different kinds of precursor cells and have plasma cells of less than 0.5 %. When the ratio of full length isoform was higher than the Δ7 isoform in these patients, it was only above 1-2 times, which was far lower than previously reported[6]. This might be due to bone marrow cells expressing Δ7 isoforms at a high level more than the full length ones. Therefore, plasma cells, which are a cell population that should express a high level of full length isoforms, were diluted with other cells.

Blimp-1 was found originally in plasma cells, but not in other stages or non B cells. By using a more sensitive method, Blimp-1 has been found to express in many cell types including premature and mature B cell lines (unpublished data). The finding of Blimp-1 at the stage preceding plasma cells is of interest, especially when investigating the ratio of full length and Δ7 isoforms that expressed at each stage of development. This study postulates if the Δ7 isoform was non functional, more of them would be expected when unwanted. It also is possible that the level of increasing full length isoform determines the function of Blimp-1. It has been reported that the steady state mRNA encoding Δ7 showed an expression of about 10 fold less than the encoding full length protein[6]. Therefore, merely a higher amount of full length than Δ7 isoform might not be sufficient to drive the function of Blimp-1, but a certain high level of full length is needed. As Zinc fingers 1 and 2 are important in the association of Blimp-1 with the co-repressor involved in catalyzing histone modification, such as G9a and HDAC1/2, the short Blimp-1Δexon7 protein has been demonstrated as incapable of binding with G9a or HDAC1/2[13].

An aberrant splicing product of the zinc finger protein, IKaros, fails to bind DNA and blocks normal Ikaros function by dimerization[7]. This functional interference raises a question as to whether this would apply to this Blimp-1 isoform. Preliminary results from this study will help to elucidate further on how Blimp-1 isoforms interact with each other and their target genes and how the expression of these isoforms might be altered in neoplastic transformation.

This study compared the levels of full length and Δ7 isoforms in primary leukemic and lymphoma cells. Blimp-1 mRNA isoforms were found to have abnormal expressions in both quality and quantity in some of the leukemia and lymphoma patients. The difference in the expression ratio of full length to Δ7 Blimp-1 isoforms was compared among the patient group, and might be involved in malignancy in some cases. The upstream and downstream regulation of Blimp-1 isoform expression will be investigated further. Preliminary results from this study will help to elucidate further on how Blimp-1 isoforms interact with each other and their target genes and how the expression of these isoforms might be altered in neoplastic transformation.

Acknowledgement

We would like to give special thanks to Dr. Chainarong Tunyaplin, from Katherine Calame's Laboratory, Columbia University College of Physicians and Surgeons, USA, Dr. Anneliese Schimpl from the Institute of Virology and Immunobiology, University of Wuerzburg, Germany, and Dr. Andreas Jung from Institute of Pathology, University of Munich, Germany, for their expertise and valuable advice.

References

1. **Turner CA Jr, Mack DH, Davis MM**. Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells. Cell 1994;77:297-306.
2. **Calame KL, Lin K-I, Tunyaplin C**. Regulatory mechanism that determine the development and function of plasma cells. Annu Rev Immunol 2003;21:205-30.
3. **Chang DH, Angelin-Duclos C, Calame K**. Blimp-1: trigger for differentiation of myeloid lineage. Nat Immunol 2000;1:169-76.
4. **Martins GA, Cimmino L, Shapiro-Shelef M, et al.** Transcriptional repressor Blimp-1 regulates T cell homeostasis and function. Nat Immunol 2006;7:457-65
5. **Kallies A, Nutt SL**. Terminal differentiation of lymphocytes depends on Blimp-1. Curr Opin Immunol 2007;19:156-62.
6. **Tunyaplin C, Shapiro MA, Calame KL**. Characterization of the B lymphocyte-induced maturation protein-1 (Blimp-1) gene, mRNA isoforms and basal promoter. Nucleic Acids Res 2000; 28:4846-55.
7. **Tabayashi T, Ishimaru F, Takata M, et al**. Characterization of the short isoform of Helios overexpressed in patients with T-cell malignancies. Cancer Sci 2007;98:182-8.
8. **Yoshida H, Matsui T, Yamamoto A, Okada T, Mori K**. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. Cell 2001;107:881-91
9. **Iwakoshi NN, Lee AH, Vallabhajosyula P, Otipoby KL, Rajewsky K, Glimcher LH**. Plasma cell differentiation and the unfolded protein response intersect at the transcription factor XBP-1. Nat Immunol 2003;4:321-9.
10. **Maestre L, Tooze R, Cañamero M, et al**. Expression pattern of XBP1(S) in human B-cell lymphomas. Haematologica 2009;94:419-22.
11. **Davies FE, Dring AM, Li C, et al**. Insights into the multistep transformation of MGUS to myeloma using microarray expression analysis. Blood 2003;102:4504–511
12. **Ocaña E, González-García I, Gutiérrez NC, Mora-López F, Brieva JA, Campos-Caro A**. The expression of PRDI-BF1 beta isoform in multiple myeloma plasma cells. Haematologica 2006;91:1579-80.
13. **Morgan MA, Mould AW, Li L, Robertson EJ, Bikoff EK**. Alternative splicing regulates Prdm1/Blimp-1 DNA binding activities and corepressor interactions. Mol Cell Biol 2012;32:3403-413.

การแสดงออกของ mRNA ไอโซฟอร์มของทรานสคริปชั่นแฟคเตอร์ Blimp-1 ในโมโนนิวเคลียร์เซลล์ในไขกระดูกที่แยกจากผู้ป่วยมะเร็งเม็ดเลือดขาวและต่อมน้ำเหลือง

เจษฎา แก้วรากมุก,[1] ลัดดาวัลย์ เหล่ามานิต,[1] มงคล ปัญยศ,[1] พิมพ์ลักษณ์ เจริญขวัญ,[2] วีระชัย นาวารวงศ์,[3] และ วิไลวรรณ เพชรโสภณสกุล[1]
[1]ภาควิชาจุลชีววิทยา, [2]ภาควิชากุมารเวชศาสตร์, [3]ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

วัตถุประสงค์ เพื่อศึกษาถึงการแสดงออกของ Blimp-1 mRNA isoform ใน mononuclear cell ที่แยกจากไขกระดูกของผู้ป่วยมะเร็งเม็ดเลือดขาวและต่อมน้ำเหลือง

วิธีการ ตัวอย่างไขกระดูกจำนวน 23 รายได้จากผู้ป่วยมะเร็งเม็ดเลือดขาวและต่อมน้ำเหลืองที่มารักษาที่โรงพยาบาลมหาราชนครเชียงใหม่ ทำการปั่นแยก mononuclear cell โดยใช้วิธี Ficoll-Hypaque gradient แล้วตรวจหาการแสดงออกของยีน Blimp-1 ไอโซฟอร์ม ชนิดสมบูรณ์ (full length) กับชนิดที่มีการขาดหายไปของ exon 7 (Δ7) โดยวิธี RT-PCR และใช้โปรแกรม Molecular Analysis ในการวัดความเข้มของ PCR product ที่เกิดขึ้น จากนั้นเปรียบเทียบสัดส่วนการแสดงออกของ Blimp-1 ไอโซฟอร์ม ทั้งสอง โดยมี GAPDH เป็นตัวควบคุม

ผลการศึกษา พบการแสดงออกที่ผิดปกติไปของ Blimp-1 ไอโซฟอร์ม ในผู้ป่วยบางราย ผู้ป่วยส่วนใหญ่จะพบไอโซฟอร์มทั้ง 2 ชนิดโดยพบว่ามีชนิด Δ7 มากกว่าชนิด full length แต่มีผู้ป่วยอยู่ 4 รายที่ให้สัดส่วนกลับตรงกันข้าม โดย 2 รายเป็นผู้ป่วยมะเร็งเม็ดเลือดขาวชนิด ALL ส่วนอีก 2 ราย เป็นชนิด AML และมะเร็งต่อมน้ำเหลือง มีผู้ป่วยมะเร็งต่อมน้ำเหลืองชนิด NHL 2 ราย ที่ยังไม่ได้รับการรักษากลับพบเฉพาะ Δ7 ชนิดเดียว ที่น่าสนใจกว่านั้นคือมีผู้ป่วย ALL 1 รายที่ไม่พบทั้ง 2 ไอโซฟอร์ม แต่กลับพบไอโซฟอร์มใหม่ซึ่งเป็น PCR product ที่มีขนาด 1,150 bp นอกจากนี้ยังพบความผิดปกติของ XBP-1 ซึ่งเป็นยีนที่ถูกควบคุมโดย Blimp-1 โดย XBP-1 เกี่ยวข้องในการควบคุมการหลั่งอิมมูโนโกลบินและจะตอบสนองเมื่ออยู่ในสภาวะ ER stress

สรุปผลการวิจัย การศึกษานี้แสดงให้เห็นถึงรูปแบบที่แสดงออกของยีน Blimp-1 ไอโซฟอร์ม ชนิดสมบูรณ์และชนิดที่มีการขาดหายไปของ exon 7 ในผู้ป่วยมะเร็งเม็ดขาวและต่อมน้ำเหลือง นอกจากนี้ยังพบไอโซฟอร์ม ใหม่ซึ่งต่างจาก 2 ไอโซฟอร์มที่ศึกษาในผู้ป่วยรายหนึ่งซึ่งเป็นไอโซฟอร์มที่ยังไม่เคยมีรายงานมาก่อน เนื่องจาก Δ7 เป็นไอโซฟอร์มที่ขาดบริเวณที่ใช้จับกับ DNA ดังนั้นการแสดงออกที่สูงกว่าปกติอาจเป็นสาเหตุหนึ่งที่เกี่ยวข้องกับการเปลี่ยนแปลงไปเป็นเซลล์มะเร็งได้ **เชียงใหม่เวชสาร 2559;55(ฉบับเสริม 1):67-74.**

คำสำคัญ: Blimp-1 ไอโซฟอร์ม เดลต้าเซเว่นไอโซฟอร์ม ไขกระดูก ผู้ป่วยมะเร็งเม็ดเลือดขาว มะเร็งต่อมน้ำเหลือง