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EBF1 acts as a powerful repressor of Blimp-1 gene expression in immature B cells

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ABSTRACT

The transcription factor, early B cell factor 1 (EBF1) with an atypical zinc-finger and helix-loop-helix motif, is essential for development and differentiation of lymphocytes. In mice, EBF1 is involved in the generation of pre-pro B cells (the first specified progenitors of B cells) from common lymphoid progenitors (CLPs) and transcription regulations of various genes involved in B cell-development, for instance, mb-1 and Pax5. During B lymphopoiesis, interestingly, EBF1 is detected throughout from CLPs to mature B cells. However, in immature B cells, the physiological role of EBF1 remains to be elucidated. Here, by analyzing EBF1-deficient DT40 cells, *EBF1*$^{-/-}$, generated by us, we show that EBF1-deficiency caused significant increases (to ~800%) in both mRNA and protein levels of B lymphocyte-induced maturation protein-1 (Blimp-1), the master gene for plasma cell differentiation. In addition, both transcription and protein synthesis of Blimp-1 were remarkably down-regulated (to ~20%) by re-expression (over-expression) of EBF1. Chromatin immunoprecipitation assay revealed that EBF1 binds to proximal 5′-upstream regions around two putative EBF1 binding motifs of the gene *in vivo*. These results suggest that EBF1 takes part in transcriptional regulations of the Blimp-1 gene in immature B cells, and may play a key role in B cell differentiation. This is the first report on a novel EBF1 function in immature B cells as a powerful repressor of Blimp-1 gene expression.

1. Introduction

In vertebrates, the normal progression of B cell-development requires various specific transcription factors, such as E2A [1], early B cell factor 1 (EBF1) [2], Ikaros family [3,4], paired box gene 5 (Pax5) [5], PU.1 [6] and so on [7,8]. EBF1, a transcription factor with zinc-finger and helix-loop-helix domains, is essential for development of B cells [9,10]. During B lymphopoiesis, EBF1 is first detected in common lymphoid progenitors (CLPs) [8]. Gene knockout studies in mice have revealed that EBF1 is required for the generation of pre-pro B cells (the first specified progenitors of B cells) from CLPs and regulates expressions of various genes involved in development of B cells, for instance, mb-1 and Pax5 [2,11,12]. Since EBF1 induces DNA demethylation and chromatin remodeling of mb-1, EBF1-deficient cells fail to express the surface immunoglobulin, a typical B cell surface marker [2,9]. EBF1 also activates Pax5 through chromatin remodeling at the Pax5 locus [9,13]. Therefore, it is well known that EBF1-mediated DNA demethylation and chromatin remodeling are common mechanisms for transcriptional regulations of the B cell-development-related genes [9,14]. Besides the transcription activation of various genes like those, EBF1 also suppresses some B cell-development-related gene expressions. For example, EBF1 inhibits expressions of Id2 and Id3 genes, resulting in normal B cell-development [15]. Recently, a genome-wide chromatin immunoprecipitation (ChIP) sequencing analysis in early-stage B cells showed that EBF1 regulates B cell gene networks by activation, repression and transcription-independent poising of chromatin [16]. Interestingly, the expression of EBF1 is sustained throughout development of B cells (from CLPs to mature B cells) after the observed developmental block in knockout mice [8]. Very recently, conditional inactivation studies of EBF1 revealed that it is required for the proliferation, survival and signaling of pro-B cells and mature B cells [17]. However, physiological functions of EBF1 beyond the developmental arrest in knockout mice, especially at immature B stages, remain poorly understood.

Gene targeting techniques in the chicken immature B cell line DT40 [18] are excellent methods to study physiological roles of transcription factors in immature B cells [19,20]. Using the techniques, we revealed not only that histone deacetylase-2 regulates transcriptions of IgM H- and L-chain genes via controlling gene expressions of EBF1, Pax5, Ikaros, Aiolos and E2A, but also that EBF1 and Aiolos down-regulate expressions of IgM H and L-chain genes, and E2A up-regulates expressions of these two genes [21]. Moreover, E2A was involved in a fine control of apoptosis mediated

Abbreviations: Ab, antibody; AID, activation-induced deaminase; Bach2, BTB and CNC homology 2; Bcl-6, B cell lymphoma-6; BCR, B cell receptor; Blimp-1, B lymphocyte-induced maturation protein-1; ChIP, chromatin immunoprecipitation; CLP, common lymphoid progenitor; EBF1, early B cell factor 1; IRF-4, interferon regulatory factor-4; OBF-1, origin binding factor-1; Pax5, paired box gene 5; RACE, rapid amplification of cDNA ends; XBP-1, X-box binding protein-1.

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by the B cell receptor (BCR) signaling via transcriptional regulation of survivin, inhibitor of apoptosis-2 and caspase-8 genes [22]. We also revealed that Aiolos and Helios regulated apoptotic cell death mediated by the BCR stimulation through transcriptional regulation of protein kinase Cs [23,24].

To understand the role of EBF1 in immature B cells in more detail, we studied the participation of EBF1 in the transcriptional regulation of various lymphocyte-specific transcription factors in EBF1-deficient DT40 mutant, *EBF1*$^{-/-}$. The results obtained revealed that EBF1 acts as a negative modulator for the gene expression of B lymphocyte-induced maturation protein-1 (Blimp-1), the master gene of plasma cell differentiation, in immature B cells.

2. Materials and methods

2.1. Materials

Bovine aprotinin, monoclonal anti-Flag M1 and M2 antibodies (Abs) (Sigma, St. Louis, MO, USA), PMSF (Wako, Osaka, Japan), protein G agarose/salmon sperm DNA (Millipore, Billerica, MA, USA) were obtained. Monoclonal anti-Blimp-1 Ab (Cell Signaling Technology, Inc., Danvers, MA, USA), monoclonal anti-β-actin Ab (Abcam, Cambridge, UK) and horseradish peroxidase-conjugated anti-mouse or rabbit immunoglobulin (DAKO, Inc., Glostrup, Denmark) were used.

2.2. Cell cultures, semiquantitative RT-PCR and immunoblotting

Generation of EBF1-deficient DT40 cells, *EBF1*$^{-/-}$, was described in our previous report [21]. DT40 cells and all subclones were cultured as described [21–26]. Semiquantitative RT-PCR and immunoblotting were carried out as described [21–26].

2.3. Re-expression study

To obtain the Flag-tagged EBF1 expression vector, a chicken EBF1 cDNA fragment of full-length was generated by PCR amplification using PrimeSTAR HS DNA polymerase (Takara Bio, Shiga, Japan), and inserted into pApuro, carrying the chicken β-actin promoter upstream of the cloning site and a marker gene (the puro-resistant gene) under the control of the SV40 promoter [23]. The *EBF1*$^{-/-}$ cells were transfected with the vector using electroporation and the transfectant clones were selected in the presence of puromycin (0.4 μg/ml).

2.4. 5′-Rapid amplification of cDNA ends (5′-RACE)

Transcription initiation site of chicken Blimp-1 was determined by 5′-RACE using a 5′-RACE Kit (Takara Bio, Shiga, Japan) according to manufacturer's instructions. The oligonucleotide 5′-CTCATTAAAGCCGTC-3′ was used as a RT-primer. The primers used in the primary PCR reaction were RACE-1 primers, and those in the second PCR reaction were RACE-2 primers (Supplementary Table S1).

2.5. ChIP assay

ChIP assay was performed using Chromatin Immunoprecipitation Assay Kit (Millipore). Preparation of cell lysates and immunoprecipitation with anti-Flag M2 Ab (or anti-Flag M1 Ab as a negative control) were carried out as described [27,28]. Immunoprecipitated and input DNAs were analyzed by PCR using appropriate primers (ChIP-1 and -2, see Supplementary Table >S1) corresponding to the 5′-upstream region of the chicken Blimp-1 gene. Primer ChIP-NC corresponding to intron 1 of the gene was

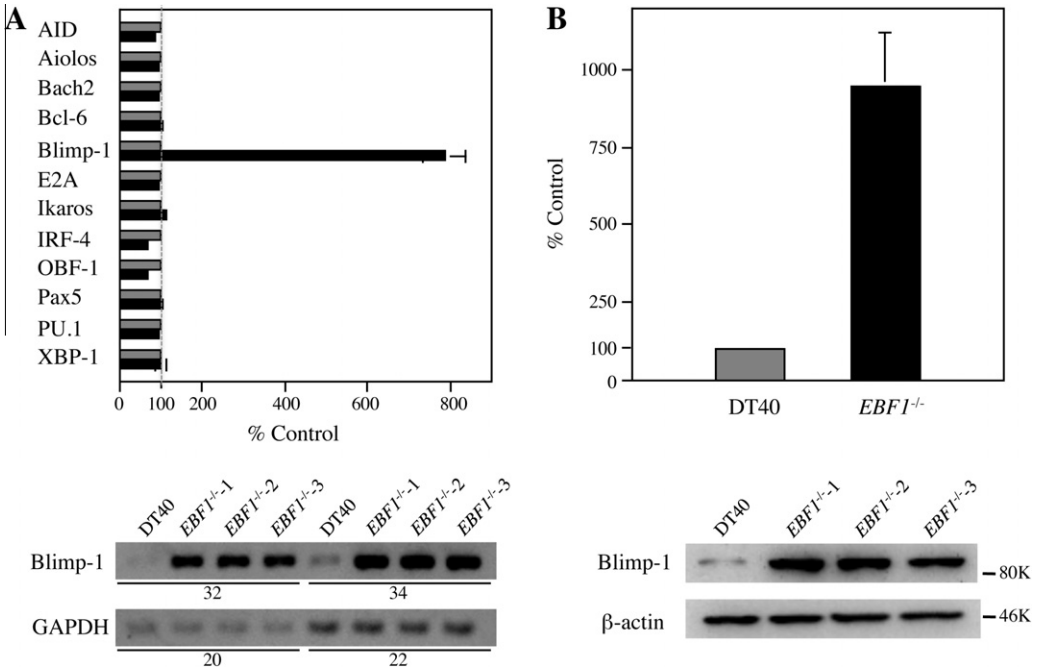

Fig. 1. Drastic up-regulation of Blimp-1 expression in EBF1-deficient DT40 cells. (A) Semiquantitative RT-PCR. Total RNAs were extracted from DT40 and three independent *EBF1*$^{-/-}$ clones 1–3, and mRNA levels of various B cell-development-related factors were determined by semiquantitative RT-PCR using appropriate primers listed in Supplementary Table S1. Chicken GAPDH gene was used as internal control. Data calibrated with the internal controls are indicated as percentages of control values (100%) obtained from DT40 (gray bars), and represent the averages of three independent *EBF1*$^{-/-}$ clones 1–3 (solid bars). Error bars indicate standard deviation. Typical semiquantitative RT-PCR profiles of Blimp-1 are shown. Cycle numbers of PCR are shown below the panels. (B) Immunoblotting. Whole proteins were isolated from DT40 and three independent *EBF1*$^{-/-}$ clones, and subjected to 10% SDS–PAGE followed by immunoblotting using Ab for Blimp-1. β-Actin was used as controls. Data are indicated as percentages of control values (100%) obtained from DT40 cells (gray bar), and represent the averages of three independent *EBF1*$^{-/-}$ clones (solid bar). Error bars indicate standard deviation. Typical immunoblotting patterns are shown.

used as a negative control. PCRs were carried out at 96 °C for 20 s, 55 °C for 30 s and 72 °C for 30 s, for 32 ~ 36 cycles, and stopped before reaching the plateau. PCR products were subjected to 1.5% agarose gel electrophoresis and analyzed using a luminescent image analyzer LAS-1000plus.

3. Results

3.1. EBF1-deficiency causes drastic up-regulation of Blimp-1 gene expression

To know effects of EBF1-deficiency on gene expressions of various B cell-development-related factors; activation-induced deaminase (AID), Aiolos, BTB and CNC homology 2 (Bach2), B cell lymphoma-6 (Bcl-6), Blimp-1, E2A, Ikaros, interferon regulatory factor-4 (IRF-4), origin binding factor-1 (OBF-1), Pax5, PU.1 and X-box binding protein-1 (XBP-1), we carried out semiquantitative RT-PCR on total RNAs prepared from DT40 and three independent *EBF1*$^{-/-}$ clones (Fig. 1A). Remarkably, in *EBF1*$^{-/-}$, the transcription of the Blimp-1 gene was drastically up-regulated (to ~800%), but those of other factors were not appreciably changed. Next, we examined influences of EBF1-deficiency on the protein level of Blimp-1 by immunoblotting analysis using rabbit anti-human Blimp-1 Ab (Fig. 1B). Chicken Blimp-1 gave a single positive band of about 90 K in length with the Ab. Quantitative data obtained from three independent *EBF1*$^{-/-}$ clones were indicated as percentages of control values obtained from the wild-type DT40. As expected, the EBF1-deficiency caused drastic increases in protein level of Blimp-1 (to ~800%), coincident with the result on the mRNA level. To further examine effects of EBF1-deficiency on B cell differentiation, we analyzed expressions of surface IgM and CD45 as markers for B cell differentiation on the cell surface by flow cytometry and secreted IgM levels in culture media by immunoblotting (Supplementary Fig. S1). The lack of EBF1 caused insignificant influences on expressions of these proteins. Thus, the remarkable up-regulation of Blimp-1 mediated by EBF1-deficiency resulted in no effect in B cell differentiation.

3.2. Re-expression of EBF1 causes remarkable down-regulation of Blimp-1 gene expression in *EBF1*$^{-/-}$

To confirm the role of EBF1 in transcription regulation of the Blimp-1 gene, re-expression study was performed using EBF1 expression vector. Two resultant transfectant clones *EBF1*$^{-/-}$/EBF1-1 and -2, which express EBF1 genes in large excess (~500%) compared with the wild-type DT40 (Fig. 2A) and synthesize definite amounts of Flag-tagged EBF1 (Fig. 2B), were used to study the complementation for the enhanced Blimp-1 gene expression in *EBF1*$^{-/-}$.

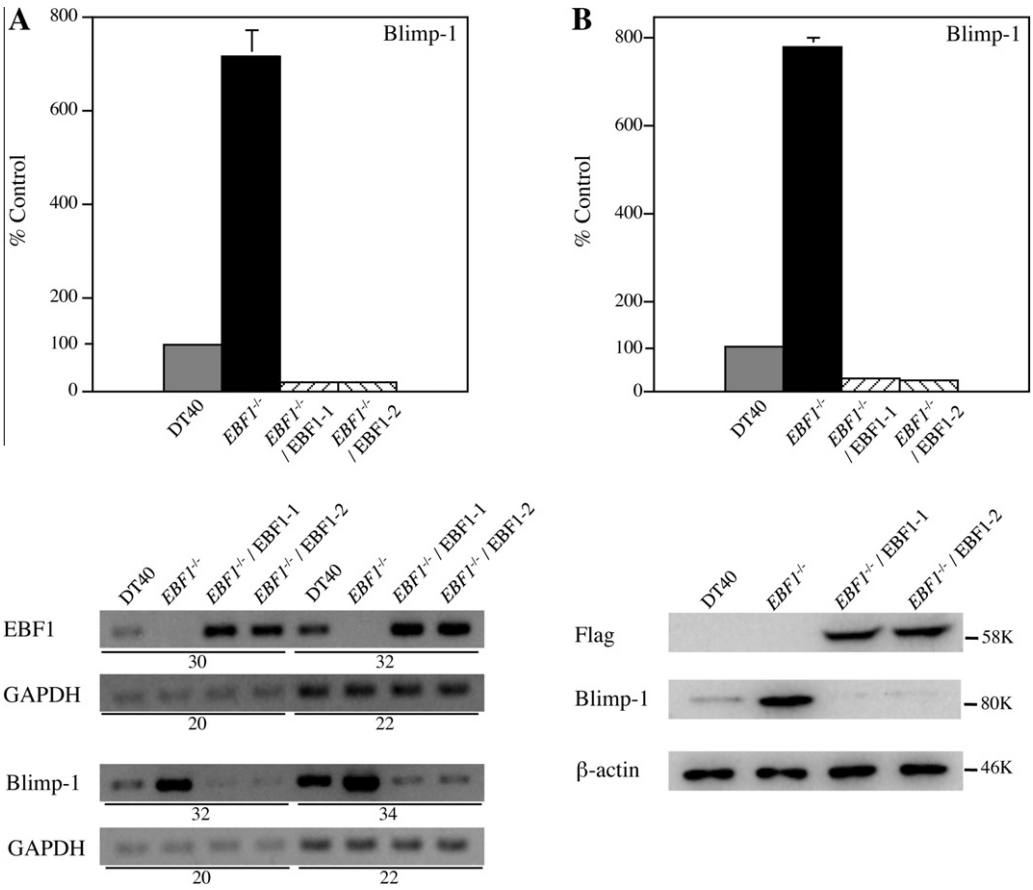

Fig. 2. Re-expression study of EBF1. (A) Influences of re-expression of EBF1 on Blimp-1 expression. Total RNAs were extracted from DT40 (gray bar), *EBF*$^{-/-}$ (solid bar) and two independent *EBF1*$^{-/-}$/EBF1 clones -1 and -2 (striped bars), and mRNA levels of Blimp-1 and EBF1 were determined by semiquantitative RT-PCR using appropriate primers listed in Supplementary Table S1. Chicken GAPDH gene was used as internal control. Data for Blimp-1 calibrated with the internal controls are indicated as percentages of control values (100%) obtained from DT40, and represent the averages of three separate experiments. Error bars indicate standard deviation. Two transfectant clones *EBF1*$^{-/-}$/EBF1-1 and -2 express EBF1 genes in large excess (~500%) compared with the wild-type DT40 (100%) (graph not shown). Typical semiquantitative RT-PCR profiles are shown. Cycle numbers of PCR are shown below the panels. (B) Influences of re-expression of EBF1 on protein level of Blimp-1. Whole proteins were isolated from DT40 (gray bar), *EBF1*$^{-/-}$ (solid bar) and two independent *EBF1*$^{-/-}$/EBF1 clones (striped bars) and subjected to 10% SDS–PAGE followed by immunoblotting using Abs for Blimp-1. Flag-tagged EBF1 was detected using monoclonal anti-Flag M2 Ab. β-Actin was used as controls. Data for Blimp-1 are indicated as percentages of control values (100%) obtained from DT40 cells, and represent the averages of three separate experiments. Error bars indicate standard deviation. Typical immunoblotting patterns are shown.

Both transcription and protein synthesis levels of Blimp-1 were remarkably down-regulated (to ~20%) in two *EBF1*$^{-/-}$/EBF1 clones (Fig. 2A and B), suggesting that EBF1 negatively regulates gene expression of Blimp-1 in immature B cells.

3.3. EBF1 binds to the 5′-flanking region of Blimp-1 gene

We examined by ChIP assay whether or not EBF1 interacts with regulatory region of the Blimp-1 gene (Fig. 3). First, to determine the transcription initiation site of the chicken Blimp-1 gene, we carried out 5′-RACE method (Fig. 3A). Data obtained revealed that the transcription initiation site G was located at 66 nucleotides 5′-upstream from the translation start codon ATG. A putative TATA-like box (GATTAA) was located at positions −23 ~ −28. Moreover, two putative EBF1 binding motifs were found within positions −500 ~ −680, respectively. Next, we carried out ChIP assay using anti-Flag Abs (Fig. 3B). Cross-linked chromatins were co-precipitated from lysates of *EBF1*$^{-/-}$ and *EBF1*$^{-/-}$/EBF1, respectively, with anti-Flag Ab M2. Anti-Flag Ab M1 used as a negative control could not bind to the Flag-tagged EBF1 with N-terminus Met. Precipitated chromatins were amplified by PCR using primers ChIP-1 and -2 (Fig. 3A and Supplementary Table S1) for the 5′ proximal regions of the Blimp-1 gene surrounding the motifs. Amplified DNA fragment could be detected in *EBF1*$^{-/-}$/EBF1 but not in *EBF1*$^{-/-}$ using ChIP-1 and -2 primers (Fig. 3B). Thus, EBF1 binds to the 5′-flanking regions of the Blimp-1 gene surrounding two putative EBF1 binding motifs *in vivo*, suggesting that gene expression of Blimp-1 is certainly regulated by EBF1 in immature B cells.

4. Discussion

Our findings obtained in this study reveal a new role of EBF1 in the regulation of Blimp-1 gene expression in immature B cells. As well known, normal B cell-development requires numerous transcription factors [1–8]. Among them, Blimp-1, a transcription factor with five zinc-fingers, controls the terminal differentiation of mature B cells to plasma cells [29,30]. Gene expression of Blimp-1 is particularly high in plasma cells but low or very low in other developmental stages of B cells [31,32]. Moreover, the B cell-specific deletion of Blimp-1 in mice leads to failure in generation of plasma cells [32], and ectopic expression of Blimp-1 is sufficient to induce plasma cell differentiation [33]. To promote plasma cell differentiation, Blimp-1 inhibits growth of B cells by repression of c-myc gene expression, and further represses function of B cells by down-regulation of Pax5 gene expression [34,35]. Since Blimp-1 plays critical roles as the master regulator in plasma cell differentiation, its gene expression should be strictly regulated during B cell differentiation. In fact, in early stage of B cell activation, Pax5 promotes gene expression of Bach2, and Bach2 collaborates with Pax5 and Bcl-6 to suppress Blimp-1 gene expression [36,37]. On the other hand, in late stage of the activation, decreased Bach2 level following diminution of Pax5 and suppression of Bcl-6 mediated by IRF-4 causes release of Blimp-1 from the repression [37]. Increased Blimp-1 level promotes plasma cell differentiation by repressing Pax5 expression and enhancing XBP-1 expression [35,38,39]. Thus, Blimp-1 gene expression is precisely regulated by the genetic network which consists of plural transcription factors.

Here, we show that EBF1 participates in the genetic network for B cell differentiation through the transcription regulation of Blimp-1. To clarify *in vivo* participatory roles of EBF1 in immature B cells, we first studied gene expressions of various factors related to B cell-development (Fig. 1). Surprisingly, both mRNA and protein levels of Blimp-1 were dramatically enhanced in *EBF1*$^{-/-}$ (to ~800%). However, EBF1-deficiency showed insignificant influence on the gene expression of IRF-4, an important activator of Blimp-1 transcription. These data suggested that IRF-4 was not involved

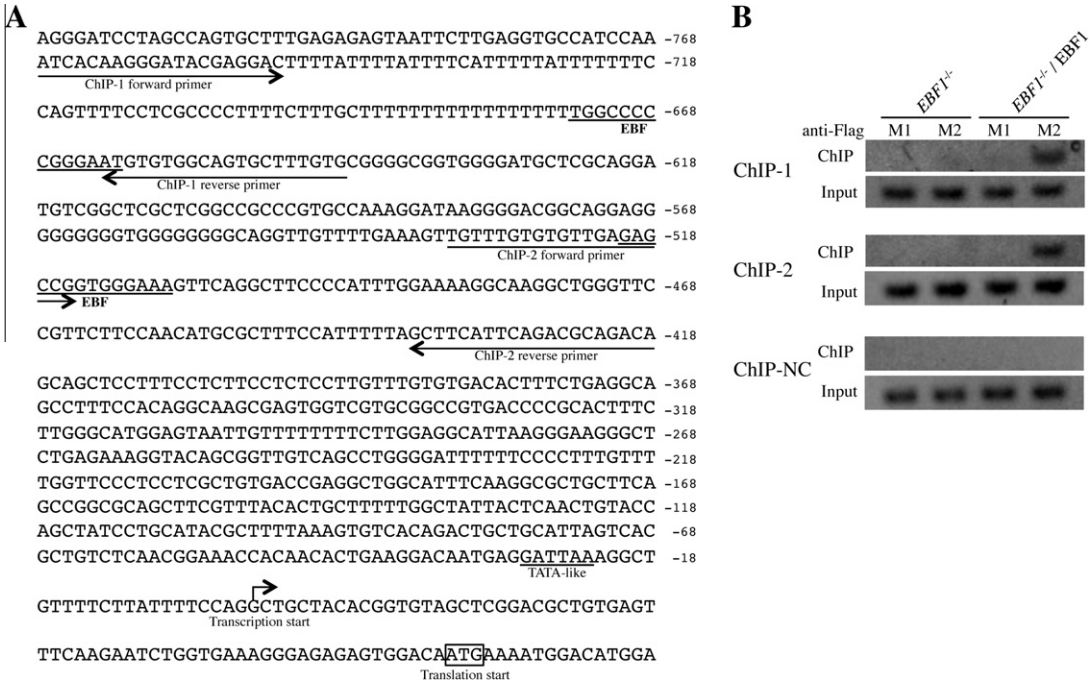

Fig. 3. Interaction of EBF1 with 5′-flanking region of chicken Blimp-1 gene. (A) Schematic diagram for the 5′-flanking region of chicken Blimp-1 gene. Two putative EBF1 binding motifs and a putative TATA-like box are underlined. The transcription start site of Blimp-1 gene determined by 5′-RACE is indicated by an arrow. The translation start codon ATG is enclosed by an open box. Forward- and reverse-primers (ChIP-1 and -2) used for PCR in ChIP assay are shown. (B) EBF1 interacts with the 5′-flanking region surrounding the putative EBF1 binding motifs of Blimp-1 gene. The cross-linked chromatins from cell lysates of *EBF1*$^{-/-}$ and *EBF1*$^{-/-}$/EBF1 were co-precipitated by monoclonal anti-Flag M2 Ab. Monoclonal anti-Flag Ab M1 was used as a negative control. After decross-linking, co-precipitated chromatins and input samples were amplified by PCR using primers ChIP-1, -2 and -NC. PCR products were analyzed by 1.5% agarose gel electrophoresis. Typical patterns are shown.

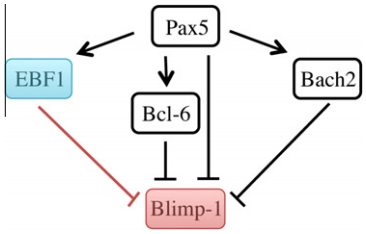

Fig. 4. A model for the Blimp-1 gene regulatory network in immature B cells.

in the up-regulation of Blimp-1 in *EBF1*$^{-/-}$. In addition, overexpression of EBF1 showed that both transcription and protein synthesis of Blimp-1 were remarkably down-regulated (to ~20%) (Fig. 2). Moreover, ChIP assay revealed that EBF1 binds to the 5′-flanking region of chicken Blimp-1 gene surrounding two putative EBF1 binding motifs *in vivo*. However, we could not determine the correct EBF1 binding site because of their close proximity. EBF1 probably binds to one or both of the motifs. Because a Pax5-binding site was also present near position −1550 within 5′-flanking region of Blimp-1 gene [40], the region would play important roles as regulatory elements for the gene. These data, together, suggested that EBF1 acts as a momentous repressor for Blimp-1 gene expression in immature B cells. It has been well known that expressions of Pax5 and Bcl-6 are down-regulated by Blimp-1 during plasma cell differentiation, and thereby plasma cell differentiation becomes possible [39,41]. Although the drastic increase in Blimp-1 expression was observed in *EBF*$^{-/-}$ cells, EBF1-deficiency showed insignificant effects even on gene expressions of Pax5 and Bcl-6, and differentiation of DT40 cells (Fig. 1A and Supplementary Fig. S1). Then, the remarkable up-regulation of Blimp-1 in *EBF*$^{-/-}$ resulted in no effect on B cell differentiation. These data suggest that there are unknown regulation systems, for example, EBF-deficiency itself should prevent DT40 cells from differentiation and/or Blimp-1 gene expression should be strictly regulated in immature B cells to prevent unexpected differentiation.

In DT40, a chicken immature B cell line, Pax5 up-regulates gene expressions of EBF1, Bcl-6 and Bach2, and down-regulates that of Blimp-1 ([42], our unpublished data). Very recently, it was reported that Bcl-6 suppresses Blimp-1 gene expression in DT40 [43]. Our findings, combined with these results, suggest that EBF1, which is up-regulated by Pax5, plays important roles in immature B cells through down-regulation of Blimp-1 gene expression (Fig. 4). However, the more detailed mechanisms of participation of EBF1 in B cell differentiation through suppression of Blimp-1 expression should be elucidated in the future. Albeit, this is the first report on a novel function of EBF1 as a powerful repressor of Blimp-1 gene expression. Further, our results may significantly help in the understanding of the mechanisms specific for differentiation of immature B cells and molecular mechanisms for plasma cell differentiation involved in lymphoid malignancies.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.05.099.

References

[1] Y. Zhuang, P. Soriano, H. Weintraub, The helix-loop-helix gene E2A is required for B cell formation, Cell 79 (1994) 875–884.
[2] H. Lin, R. Grosschedl, Failure of B-cell differentiation in mice lacking the transcription factor EBF, Nature 376 (1995) 263–267.
[3] P. Urbanek, Z.Q. Wang, I. Fetka, E.F. Wagner, M. Busslinger, Complete block of early B cell differentiation and altered patterning of the posterior midbrain in mice lacking Pax5/BSAP, Cell 79 (1994) 901–912.
[4] K.L. Anderson, K.A. Smith, K. Conners, S.R. McKerche, R.A. Maki, B.E. Torbett, Myeloid development is selectively disrupted in PU.1 null mice, Blood 91 (1998) 3702–3710.
[5] C.A. Klug, S.J. Morrison, M. Masek, K. Hahm, S.T. Smale, I.L. Weissman, Hematopoietic stem cells and lymphoid progenitors express different Ikaros isoforms, and Ikaros is localized to heterochromatin in immature lymphocytes, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 657–662.
[6] J.H. Wang, N. Avitahl, A. Cariappa, C. Friedrich, T. Ikeda, A. Renold, K. Andrikopoulos, L. Liang, S. Pillai, B.A. Morgan, K. Georgopoulos, Aiolos regulates B cell activation and maturation to effector state, Immunity 9 (1998) 543–553.
[7] E. Smith, M. Sigvardsson, The roles of transcription factors in B lymphocytes commitment, development, and transformation, J. Leukoc. Biol. 75 (2004) 973–981.
[8] J. Ramirez, K. Lukin, J. Hagman, From hematopoietic progenitors to B cells: mechanisms of lineage restriction and commitment, Curr. Opin. Immunol. 22 (2010) 177–184.
[9] D. Liao, Emerging roles of the EBF family of transcription factors in tumor suppression, Mol. Cancer Res. 7 (2009) 1893–1901.
[10] K. Lukin, S. Fields, J. Hartley, J. Hagman, Early B cell factor: regulator of B lineage specification and commitment, Semin. Immunol. 20 (2008) 221–227.
[11] M. Sigvardsson, D.R. Clark, D. Fitzsimmons, M. Doyle, P. Akerblad, T. Breslin, S. Bilke, R. Li, C. Yeamans, G. Zhang, J. Hagman, Early B-cell factor, E2A, and Pax5 cooperate to activate the early B cell-specific mb-1 promoter, Mol. Cell. Biol. 22 (2002) 8539–8551.
[12] M. O'Riordan, R. Grosschedl, Coordinate regulation of B cell differentiation by the transcription factors EBF and E2A, Immunity 11 (1999) 21–31.
[13] T. Decker, M. Pasca di Magliano, S. McManus, Q. Sun, C. Bonifer, H. Tagoh, M. Busslinger, Stepwise activation of enhancer and promoter regions of the B cell commitment gene Pax5 in early lymphopoiesis, Immunity 30 (2009) 508–520.
[14] S.L. Nutt, B.L. Kee, The transcriptional regulation of B cell lineage commitment, Immunity 26 (2007) 715–725.
[15] M.A. Thal, T.L. Carvalho, T. He, H.G. Kim, H. Gao, J. Hagman, C.A. Klug, Ebf1-mediated down-regulation of Id2 and Id3 is essential for specification of the B cell lineage, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 552–557.
[16] T. Treiber, E.M. Mandel, S. Pott, I. Gyry, S. Firner, E.T. Liu, R. Grosschedl, Early B cell factor 1 regulates B cell gene networks by activation, repression, and transcription-independent poising of chromatin, Immunity 32 (2010) 714–725.
[17] I. Gyory, S. Boller, R. Nechanitzky, E. Mandel, S. Pott, E. Liu, R. Grosschedl, Transcription factor EBF1 regulates differentiation stage-specific signaling, proliferation, and survival of B cells, Genes Dev. 26 (2012) 668–682.
[18] J.M. Buerstedde, S. Takeda, Increased ration of targeted to random integration after transfection of chicken B cell lines, Cell 67 (1991) 179–188.
[19] J. Alinikula, O. Lassila, K.P. Nera, DT40 mutants: a model to study transcriptional regulation of B cell development and function, in: J.M. Buerstedde, S. Takeda (Eds.), Reviews and Protocols in DT40 Research, Springer-Verlag, Berlin, 2006, pp. 189–205.
[20] H. Kikuchi, H.K. Barman, M. Nakayama, Y. Takami, T. Nakayama, Participation of histones, histone modifying enzymes and histone chaperons in vertebrate cell functions, in: J.-M. Buerstedde, S. Takeda (Eds.), Reviews and Protocols in DT40 Research, Springer-Verlag, Berlin, 2006, pp. 225–243.
[21] M. Nakayama, H. Suzuki, N. Yamamoto-Nagamatsu, H.K. Barman, H. Kikuchi, Y. Takami, K. Toyonaga, K. Yamashita, T. Nakayama, HDAC2 controls IgM H- and L-chain gene expressions via EBF1, Pax5, Ikaros, Aiolos and E2A gene expressions, Genes Cells 12 (2007) 359–373.
[22] K. Toyonaga, H. Kikuchi, K. Yamashita, M. Nakayama, K. Chijiiwa, T. Nakayama, E2A participates in a fine control of pre-mature B-cell apoptosis mediated by B-cell receptor signaling via transcriptional regulation of survivin, IAP2 and caspase-8 genes, FEBS J. 276 (2009) 1418–1428.
[23] H. Kikuchi, K. Yamashita, M. Nakayama, K. Toyonaga, I. Tsuneyoshi, M. Takasaki, T. Nakayama, Lacking of Aiolos accelerates pre-mature B cell apoptosis mediated by BCR signaling through elevation in cytochrome *c* release, Biochim. Biophys. Acta 1793 (2009) 1304–1314.
[24] H. Kikuchi, M. Nakayama, Y. Takami, F. Kuribayashi, T. Nakayama, Possible involvement of Helios in controlling the immature B cell functions via transcriptional regulation of protein kinase Cs, Results Immunol. 1 (2011) 88–94.
[25] H. Kikuchi, Y. Takami, T. Nakayama, GCN5: a supervisor in all-inclusive control of vertebrate cell cycle progression through transcription regulation of various cell cycle-related genes, Gene 347 (2005) 83–97.
[26] H. Kikuchi, T. Nakayama, GCN5 and BCR signalling collaborate to induce pre-mature B cell apoptosis through depletion of ICAD and IAP2 and activation of caspase activities, Gene 419 (2008) 48–55.

[27] H. Kikuchi, F. Kuribayashi, N. Kiwaki, Y. Takami, T. Nakayama, GCN5 regulates the superoxide-generating system in leukocytes via controlling gp91-phox gene expression, J. Immunol. 186 (2011) 3015–3022.
[28] H. Kikuchi, F. Kuribayashi, Y. Takami, S. Imajoh-Ohmi, T. Nakayama, GCN5 regulates the activation of PI3K/Akt survival pathway in B cells exposed to oxidative stress via controlling gene expressions of Syk and Btk, Biochem. Biophys. Res. Commun. 405 (2011) 657–661.
[29] S.L. Nutt, K.A. Fairfax, A. Kallies, BLIMP1 guides the fate of effector B and T cells, Nat. Rev. Immunol. 7 (2007) 923–927.
[30] S. Crotty, R.J. Johnston, S.P. Schoenberger, Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation, Nat. Immunol. 11 (2010) 114–120.
[31] C. Angelin-Duclos, G. Cattoretti, K.I. Lin, K. Calame, Commitment of B lymphocytes to a plasma cell fate is associated with Blimp-1 expression in vivo, J. Immunol. 165 (2000) 5462–5471.
[32] M. Shapiro-Shelef, K.I. Lin, L.J. McHeyzer-Williams, J. Liao, M.G. McHeyzer-Williams, K. Calame, Blimp-1 is required for the formation of immunoglobulin secreting plasma cells and pre-plasma memory B cells, Immunity 19 (2003) 607–620.
[33] C.A. Turner Jr, D.H. Mack, M.M. Davis, Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells, Cell 77 (1994) 297–306.
[34] Y. Lin, K. Wong, K. Calame, Repression of c-myc transcription by Blimp-1, an inducer of terminal B cell differentiation, Science 276 (1997) 596–599.
[35] K.I. Lin, C. Angelin-Duclos, T.C. Kuo, K. Calame, Blimp-1-dependent repression of Pax-5 is required for differentiation of B cells to immunoglibulin M-secreting plasma cells, Mol. Cell. Biol. 22 (2002) 4771–4780.
[36] A. Muto, K. Ochiai, Y. Kimura, A. Itoh-Nakadai, K.L. Calame, D. Ikebe, S. Tashiro, K. Igarashi, Bach2 represses plasma cell gene regulatory network in B cells to promote antibody class switch, EMBO J. 29 (2010) 4048–4061.
[37] A. Kallies, S.L. Nutt, Bach2: plasma-cell differentiation takes a break, EMBO J. 29 (2010) 3896–3897.
[38] A.L. Shaffer, K.I. Lin, T.C. Kuo, X. Yu, E.M. Hurt, A. Rosenwald, J.M. Giltnane, L. Yang, H. Zhao, K. Calame, L.M. Staudt, Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program, Immunity 17 (2002) 51–62.
[39] B. Klein, K. Tarte, M. Jourdan, K. Mathouk, J. Moreaux, E. Jourdan, E. Legouffe, J.D. Vos, J.F. Rossi, Survival and proliferation factors of normal and malignant plasma cells, Int. J. Hematol. 78 (2003) 106–113.
[40] F. Mora-Lopez, E. Reales, J.A. Brieva, A. Campos-Caro, Human BSAP and BLIMP1 conform an autoregulatory feedback loop, Blood 110 (2007) 3150–3157.
[41] A. Hangaishi, M. Kurokawa, Blimp-1 is a tumor suppressor gene in lymphoid malignancies, Int. J. Hematol. 91 (2010) 46–53.
[42] K.P. Nera, P. Kohonen, E. Narvi, A. Peippo, L. Mustonen, P. Terho, K. Koskela, J.M. Buerstedde, O. Lassila, Loss of Pax5 promotes plasma cell differentiation, Immunity 24 (2006) 283–293.
[43] J. Alinikula, K.P. Nera, S. Junttila, O. Lassila, Alternate pathways for Bcl6-mediated regulation of B cell to plasma cell differentiation, Eur. J. Immunol. 41 (2011) 2404–2413.