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The SWI/SNF-like BAF Complex Is Essential for Early B Cell Development

Jinwook Choi,*1 Myunggon Ko,*1,2 Shin Jeon,* Yoon Jeon,† Kyungsoo Park,* Changjin Lee,* Ho Lee,† and Rho H. Seong*

During the process of B cell development, transcription factors, such as E2A and Ebf1, have been known to play key roles. Although transcription factors and chromatin regulators work in concert to direct the expression of B lineage-specific genes, little is known about the involvement of regulators for chromatin structure during B lymphopoiesis. In this article, we show that deletion of Srg3/mBaf155, a scaffold subunit of the SWI/SNF-like BAF complex, in the hematopoietic lineage caused defects at both the common lymphoid progenitor stage and the transition from pre–pro-B to early pro-B cells due to failures in the expression of B lineage-specific genes, such as Ebf1 and Il7ra, and their downstream target genes. Moreover, mice that were deficient in the expression of Brg1, a subunit of the complex with ATPase activity, also showed defects in early B cell development. We also found that the expression of Ebf1 and Il7ra is directly regulated by the SWI/SNF-like BAF complex. Thus, our results suggest that the SWI/SNF-like BAF complex facilitates early B cell development by regulating the expression of B lineage-specific genes.


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Abbreviations used in this article: ChIP, chromatin immunoprecipitation; CLP, common lymphoid progenitor; FO, follicular; HSC, hematopoietic stem cell; LMPP, lymphoid-primed multipotent progenitor; MZ, marginal zone; Pax5, paired box protein 5; pIpC, polyinosinic-polycytidylic acid; shRNA, short hairpin RNA.

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complex mobilizes nucleosomes by using the energy from ATP hydrolysis and is required for the expression of a number of genes (17, 18). The SWI/SNF-like BAF complex is composed of multiple subunits that are conserved through evolution. Brg1 with ATPase catalytic activity, Baf155 (also known as Srg3), Baf170, and Baf47 (also known as Snf5) are sufficient to induce remodeling activity in vitro; therefore, these subunits are considered the core subunits of the complex (17). Srg3 is a murine homolog of Swi3 in yeast and Baf155 in humans (19). Srg3/mBaf155 controls the stability of the SWI/SNF-like BAF complex and, thereby, serves as a scaffold protein (20, 21). It was also shown that the chromatin-remodeling activity is enhanced in transgenic mice overexpressing Srg3/mBaf155 (22).

It is still unknown how the process of hematopoiesis, particularly that of early lymphoid lineage differentiation, is epigenetically controlled, although there are a few studies suggesting its role during myeloid lineage differentiation (23, 24) and T cell development (25–27). In this study, we show that the SWI/SNF-like BAF complex plays a critical role in the process of early B cell lineage commitment by facilitating the expression of Ebf-1 and Il7ra.

Materials and Methods

Mice

Targeting constructs and strategy for the generation of conditional Srg3/mBaf155 knockout mice are described in Fig. 1D. Mice with loxP sites flanking exon 4 of Srg3 (Srg3lox) were backcrossed onto C57BL/6 mice for at least eight generations and were crossed with Mx1-Cre mice for deletion of Srg3 at various stages of the hematopoietic lineage. Mx1-Cre mice were purchased from The Jackson Laboratory. All mice were bred and maintained in specific pathogen-free barrier facilities at Seoul National University.

Administration of polyinosinic-polycytidylic acid

For the deletion of Srg3, 6–8-wk-old control (Srg3fl/fl or Srg3fl/+ ) and Mice with Srg3/mBaf155 (Srg3fl) were backcrossed onto C57BL/6 mice for at least eight generations and were crossed with Mx1-Cre mice for deletion of Srg3 at various stages of the hematopoietic lineage. Mx1-Cre mice were purchased from The Jackson Laboratory. All mice were bred and maintained in specific pathogen-free barrier facilities at Seoul National University.

Construction of targeting constructs

Targeting constructs and strategy for the generation of conditional Srg3/mBaf155 knockout mice are described in Fig. 1D. Mice with loxP sites flanking exon 4 of Srg3 (Srg3lox) were backcrossed onto C57BL/6 mice for at least eight generations and were crossed with Mx1-Cre mice for deletion of Srg3 at various stages of the hematopoietic lineage. Mx1-Cre mice were purchased from The Jackson Laboratory. All mice were bred and maintained in specific pathogen-free barrier facilities at Seoul National University.

Construction of targeting constructs

For sequence-specific knockdown of Srg3, targeting sequences (Srg3, 5'–CATCCTGTGTGGATTTAAA–3') and Brg1, 5'–CGTCAAG-GGTGAGATCAAC–3' were cloned into MDH1–PGK–GFP2.0 (kindly provided by Dr. Chang-Zhen Cheng, Stanford University) or MSCV–LTa (Linearized Targeting vectors) to generate two LTR–GFP–LRC targeting vectors. The LTR–GFP–LRC targeting vectors were co-transfected into 293FT cells with PGK–Neo to select homologous integration vectors. The correct integration in each homologous integration vector was confirmed by genomic DNA analysis. Brg1 and Srg3 were knocked down in primary pro-B cells by retroviral delivery of lentiviral vectors in combination with a LTR–miR30–PIG (Open Biosystems) retroviral vector (20). Total RNAs were purified from cells with TRIzol reagent, following the manufacturer (Invitrogen). Equivalent quantities of total RNA were reverse transcribed with SuperScript III (Invitrogen), and diluted cDNAs were analyzed by semi-quantitative PCR. PCR products were separated on agarose gels and visualized by ethidium bromide. SYBR Green PCR mix (Applied Biosystems) or TaqMan Master mix (Applied Biosystems) was used for quantitative real-time PCR analysis (primer sequences are available upon request), and the results were quantified with StepOnePlus (Applied Biosystems).

Western blot analysis

Whole-cell lysates were separated by SDS-PAGE and transferred to Immobilon-P membrane (Millipore). Blotted proteins were detected using Abs to SRG3, BRG1, FLAG epitope (AC-15, Sigma), or FLA epitope (M2, Sigma) in TBST (150 mM NaCl, 10 mM Tris-Cl [pH 8.0], 0.5% Tween-20) containing 5% low-fat milk. Antisera against SRG3 and BRG1 were raised from rabbits in our laboratory.

Southern blot analysis

Genomic DNA preparation and Southern blotting were done mainly as described (28). Briefly, DNA was purified from B220+CD43+B220−pro-B cells obtained from plpC-treated control and Mx1-Srg3lox mice by phenol-chloroform extraction and resuspended in 10 mM Tris (pH 8.0) and 1 mM EDTA. PCR analyses of Ig genes were performed with published primers. The PCR product of HSS3 was used as a loading control. DNA samples amplified by PCR were separated on agarose gels and visualized by ethidium bromide and transferred to Hybond-N membranes (Amersham). Southern blotting was probed with 32P-labeled oligonucleotides.

Chromatin immunoprecipitation assay

For the chromatin immunoprecipitation (ChIP) assay, a solution of 37% (v/v) formaldehyde was added to the culture medium of PD36 cell line or primary pro-B cells to a final concentration of 1% and 1% BSA, followed by washing twice with ice-cold PBS. Then, cells were lysed for 10 min with SDS lysis buffer (155 mM NH4Cl, 10 mM KHCO3, and 0.1 mM EDTA). Protein-DNA complexes were sonicated to an average size of 100–500 bp, and the sonicated cell supernatants were diluted 10-fold in dilution buffer (1% Triton X-100, 2 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl [pH 8.1], and Protease Inhibitor mixture [Roche]). Then, lysates were precleared for 1 h with salmon sperm DNA/Protein–A agarose (50% slurry), followed by incubation overnight at 4 °C with isotype-control anti-rabbit IgG (Upstate), anti-Brg1, anti-Srg3, or anti-H3K9me3 (Upstate). The beads were washed sequentially once with a low-salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl [pH 8.1], 150 mM NaCl), a high-salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl [pH 8.1], 500 mM NaCl), and LiCl washing buffer (0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl [pH 8.1]) and twice with 10 mM Tris (pH 8.0) plus 1 mM EDTA. Cross-linked chromatin–histone complexes were eluted with elution buffer (1% SDS, 1 M NaHCO3). After washing and elution steps, cross-linking was reversed by heating at 65°C for 4 h, followed by proteinase K treatment for an additional 1 h. DNA was purified with a QIAquick spin kit (QIAGEN, Seoul, Korea), and DNA was analyzed by PCR with primers within the Ebf1 or Il7ra promoter regions. The sequences of the primers used are as follows: Ebf1 promoter regions, P1, 5′-TCAGACTAGCTGAGAATCTCCCATA-3′, P2, 5′-ATGATGAGTTCCTTTGTCC-3′, P3, 5′-CTACAGCAACACGCAGTCCCT-3′, and P4, 5′-GGGCTGGAGTTTCCTTTTGT-3′; Il7ra promoter regions, P1, 5′-CACCTTGTGAACTGAAAG-3′ and P2, 5′-GAAGACGGCGCTGTATGGTCG-3′; and Gapdh intron regions, sense, 5′-TTACTTGTGGCCGCCCAGAG-3′ and antisense, 5′-GGGCTTTGATCACCTTCTCCTT-3′.

Flow cytometry and cell sorting

Single-cell suspensions were prepared from the bone marrow (femur and tibia), spleen, lymph node, and thymus by passing through a cell strainer to remove cell debris. To deplete the B cells, an equal volume of ACK RBC lysis buffer (155 mM NH4Cl, 10 mM KHCO3, and 0.1 mM EDTA) was added to the cells and incubated on ice for 5 min. Then up to 10 ml PBS plus 0.1% FBS were added to stop the lysis reaction. Cells were counted, and 1–3×106 cells were used for Ab staining. Then, cells were stained with mAbs in various combinations in 1× PBS containing 1% heat-inactivated FBS. Biotinylated Abs were revealed by PerCP- or allophycocyanin-conjugated streptavidin (BD Biosciences). Intracellular staining was performed following the manufacturer’s protocols (BD Biosciences). Flow cytometric analyses were performed using FACSCan II (BD Bioscience), and FACSARia II (BD Biosciences) was used for cell sorting. Data were analyzed using FlowJo software. The following Abs were used: biotin-conjugated mouse lineage (Lin) panel that contains anti-B220 (RA3-6B2), anti-CD5 (145-2C11), anti-Gr-1 (RB6-8C5), anti-CD11b (M1/70), and anti–Ter-119 Abs; CD34 (RAM34)-PE; CD17 (c-Kit, 2B8)-PE and PE-Cy7; CD127 (IL-7Ra, SB-199)-PE; B220 (RA3-6B2)-allophycocyanin; CD43 (57)-PE; IgM (R6-60.2 or II/41)-PE; Cy7; and –biotin; CD19 (1D3)-allophycocyanin–Cy7; CD4 (GK1.5)-PE; CD6 (53-6.7)-FITC, –biotin, and –PE; CD3 (145-2C11)-PE; TCRβ (H57-597)-allophycocyanin; Mac-1 (CD11b, M1/70)-allophycocyanin Alexa Fluor 750; c-Kit (CD117, 2B8)-allophycocyanin Alexa Fluor 750; CD44 (IM7)-biotin; CD25 (PC61.5)-allophycocyanin; and CD34 (RAM34)-allophycocyanin. B220 (RA3-6B2)-FITC and IgD (11-26)–PE were purchased from Invitrogen and Southern Biotech, respectively.
In vitro B cell differentiation assay pro-B cell culture

The murine OP9 stromal cells were maintained in α-MEM supplemented with 5% FBS. For in vitro culture, HSCs or pro-B cells were isolated from plpC-treated control and Mx1-Srg33fl/fl mice by FACSAria II (BD Biosciences) at day 5 or 10 after the final induction and cocultured (BD Biosciences) with gamma-irradiated (30 Gy) OP9 cells in RPMI 1640 supplemented with 10% FBS and cytokines. Stem cell factor (25 ng/ml), Flk2/Flt3 ligand (10 ng/ml), and IL-7 (20 ng/ml; all from PeproTech) were used for the culture of hematopoietic stem cells, and IL-7 (20 ng/ml; PeproTech) only was used for the culture of pro-B cells. After 3–6 d of culture, both suspended and adherent cells were harvested, stained with lineage markers, including Gr-1, B220, and CD19, and analyzed using a FACSCanto II (BD Biosciences).

Retroviral infection and bone marrow reconstitution

Bone marrow cells were flushed out of femurs and tibias after i.p. injection with 5-fluorouracil at 150 μg/g body weight for 4 d. RBCs were removed using ACK lysis buffer (0.15 M NH4Cl, 10 mM KHCO3, and 0.1 mM EDTA). Then, the cells were cultured overnight in complete RPMI 1640 (10% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, 50 μg/ml 2-ME, 1% Glutamax [Life Technologies], 1% nonessential amino acid [Life Technologies], and 1% sodium pyruvate [Life Technologies]) containing 5 ng/ml IL-3, 10 ng/ml IL-6, 50 ng/ml stem cell factor, and 50 ng/ml Flk3 ligand (all from PeproTech) before the initial retroviral infection. The packaging Phoenix-eco cells were transfected with a control empty vector and Brg1 short hairpin RNA (shRNA) using a standard calcium phosphate protocol to generate retroviruses. After 48 h, the viral supernatants were harvested and used to spin-infect HSC-enriched cells for 1.5 h at 2500 rpm and 30°C in the presence of 10 μg/ml polybrene. This procedure was repeated three times, followed by the injection of 2–3 × 106 infected cells via the tail vein into each recipient that was lethally gamma-irradiated with 1100 rad at 2–3 h intervals (137Cs): GC 3000 Elan; MDS Nordion.

Statistical tests

Two-tailed Student t tests were used to calculate p values where indicated; p values < 0.05 were considered statistically significant.

Results

The SWI/SNF-like BAF complex is required for normal B cell development

We first examined the expression pattern of the SWI/SNF-like BAF complex in different lineage cells. Hematopoietic progenitor-enriched populations (Lin-+) and B cells expressed Srg33fl/bag155 (hereafter referred to as Srg3) protein highly compared with erythroid and myeloid lineage cells (Fig. 1A). We also analyzed Srg3 transcript levels throughout the lymphoid lineage differentiation. Srg3 was highly expressed in HSCs (LSKs), CLPs, and pre-pro-B (Fr. A) cells (Fig. 1B). Srg3 expression was gradually increased during B cell maturation from early pro-B cells to pre-B cells. Srg1, a core subunit of the SWI/SNF-like BAF complex with ATPase activity, also showed a similar expression pattern (Fig. 1C).

Previous studies showed that the complete deletion of Srg3 resulted in embryonic lethality during the peri-implantation stage (29). To overcome this restriction and examine the function of Srg3 in hematopoietic lineage differentiation, we generated conditional knockout mice (Mx1-Srg33fl/fl) carrying a loxP-flanked exon 4 (Fig. 1D–F) and crossed them with Mx1-Cre transgenic mice, in which treatment with pIpC induced efficient gene deletion in hematopoietic lineage cells (30). After treatment with pIpC, we confirmed the deletion of Srg3 alleles in genomic DNAs (Fig. 1G). We also confirmed a drastic reduction in the protein levels in B cells purified from the bone marrow and spleen of Mx1-Srg33fl/fl mice compared with control (Fig. 1H, I). We then analyzed the effect of Srg3 deficiency on the hematopoietic system, especially B cell development, by comparing Mx1-Srg33fl/fl mice with control littermates. Srg3-deficient mice showed a drastic decrease in the percentage of B220+CD19+ B cells in the bone marrow (14.24 ± 1.212%) compared with control (26.09 ± 1.456%) (Fig. 2A). In accordance with the reduction in B cell frequency, the B cell numbers were also greatly reduced (40.94 ± 4.493 versus 17.51 ± 3.233 × 10⁶) (Fig. 2B). In the spleen, B cell generation was also defective in the absence of Srg3 (Fig. 2C). Moreover, Srg3-deficient mice showed a significant reduction in B cell numbers to half that of control mice (Fig. 2D). In addition, interestingly, the percentage of follicular (FO) B cells was significantly decreased, whereas the frequency of the marginal zone (MZ) B cells was increased in Mx1-Srg33fl/fl mice (Fig. 2E). The numbers of FO B cells were also reduced by as much as half (Fig. 2F). These results indicate that Srg3 is essential for proper B cell development in both the bone marrow and the periphery.

Impairment of B-lineage potential in Srg3-deficient lymphoid progenitors

Srg3 is expressed at the early stage of hematopoietic progenitor cells. Thus, we investigated the function of Srg3 in HSC maintenance and the development of lymphoid progenitors. The frequency of the Lin-IL-7Rα-c-Kit+Sca-1+ (LSK) subset was comparable to that of control mice. The subsets of long-term HSCs, short-term HSCs, and the LMPPs were not significantly altered in the absence of Srg3, although a mild decrease in LMPPs was observed (data not shown). The frequency and cell numbers of traditional Lin-IL-7Rα-c-KitlowSca-1low CLPs, which are the precursors of B and T cells, were not significantly altered by Srg3 deletion (Fig. 3A, 3B). The CLP population is heterogeneous, and genes involved in early B cell development, including Ebf1 and Rag1, are highly transcribed in B cell-restricted CLPs (31–34). Interestingly, CLPs highly expressing AA4.1 on the surface were significantly reduced in Mx1-Srg33fl/fl mice compared with control mice (Fig. 3C). AA4.1lo CLPs are more potent in the generation of B lineage cells, but not myeloid lineage cells, than are traditional CLPs (35, 36). To assess whether the Srg3-deficient CLPs have normal lymphoid potential, we analyzed the expression of B lineage-specific genes in these CLPs. Notably, CLPs purified from Srg3-deficient mice showed less Ebf1 expression compared with those from control mice, whereas the expression of flt3 remained unchanged (Fig. 3D). It was reported that Ebf1-deficient CLPs express very low levels of B lineage-specific genes (37). We also found that expression of B lineage-specific genes, such as Cd79a (mb-1), Cd79b (B29), Rag1, Rag2, and FoxO1, were significantly reduced in Srg3-deficient CLPs (Fig. 3E).

Pro-B cell development was defective in the absence of BAF complex

Although Srg3-deficient lymphoid progenitors showed defects in the expression of genes that are associated with the development of early B-lineage cells, a considerable number of CLPs was still detected in the absence of Srg3. Therefore, we assessed the cell specification processes in Mx1-Srg33fl/fl mice. Consistent with the lower numbers of B cells in Srg3-deficient mice, B cell development was blocked at the early stage of B-lineage cells in the bone marrow of Mx1-Srg33fl/fl mice. Analysis of Mx1-Srg33fl/fl mice showed that most of the B cells were arrested at the pro-B cell stage, resulting in a significant reduction in the frequency of pre-B cells and immature B cells compared with those from control mice (Fig. 4A, 4B). The number of pro-B cells was decreased in Mx1-Srg33fl/fl mice, even though the Srg3-deficient mice had a higher percentage of pro-B cells (Fig. 4C). To further define the defective stage in pro-B cell development in Srg3-deficient mice, we subcategorized the pro-B cells using the differential cell surface markers BP-1 and CD24 (HSA) or CD19. The proportion of CD19+...
cells was severely reduced in the pro-B cells of Mx1-Srg3 fl/fl mice (Fig. 4D). In addition, Mx1-Srg3 fl/fl mice had significantly lower frequencies of early pro-B cells (Fr. B) and late pro-B cells (Fr. C) but higher frequencies of pre–pro-B (Fr. A) cells than did the control mice (Fig. 4D, 4E). In fact, the numbers of early and late pro-B cells were also severely decreased, whereas the number of

FIGURE 1. Generation of Srg3 conditional knockout mice. (A) Western blot analysis of SRG3 expression levels from the indicated subsets in the bone marrow. Expression of β-actin was used as a loading control. Representative results of three independent experiments are shown. (B) Relative expression of Srg3 mRNA in sorted lymphoid lineage compartment in the bone marrow using TaqMan real-time PCR analysis. The expression of gapdh served as a loading control. Data are shown as mean ± SEM (n = 3). (C) Relative expression of Brg1 mRNA in sorted lymphoid lineage compartment in the bone marrow using TaqMan real-time PCR analysis. The expression of gapdh served as a loading control. Data are shown as mean ± SEM (n = 3). (D) Schematic representation of the targeting strategy. (E) Southern blot analysis of genomic DNA from wild-type (+/+) and targeted (flox/+) embryonic stem cells with a probe overlapping exon 3 reveals a different HindIII (7.5 versus 10.2 kb) fragment in the targeted cells. (F) PCR analysis of tail DNA from wild-type (+/+), heterozygous (flox/+), and homozygous (flox/flox) mice using primers in introns 3 and 4 [P1 and P2 or P3 and P4, as indicated by arrows in (D)]. (G) Representative PCR analysis of bone marrow cells after plpC treatment of control and Mx1-Srg3 fl/fl mice. (H) Western blot analysis of SRG3 expression in B220 +CD19+ B cells of bone marrow purified from plpC-treated control, Mx1-Srg3 fl/+ , and Mx1-Srg3 fl/fl mice. (I) Western blot analysis of SRG3 expression in splenic B220 +CD19+ B cells purified from plpC-treated control and Mx1-Srg3 fl/+ mice. B220+, B lineage cells; Bh, BamHI; DT-A, diphtheria toxin A-chain gene; Gr-1, Mac-1, myeloid lineage cell population; H, HindIII; Lin–, hematopoietic progenitor-enriched population; neo, neomycin-resistance gene; Ter-119+, erythroid lineage cells.
pre–pro-B cells remained largely unchanged (Fig. 4F). In previous studies, Fr. A population was shown to be heterogeneous and to contain AA4.1+ and AA4.12 cells. AA4.1+ Fr. A cells are subdivided into A1 and A2, and they are considered the earliest B-lineage population (7). Mx1-Srg3 fl/fl mice showed significantly fewer AA4.1+ Fr. A cells compared with the control mice (Fig. 4G). Overall, Srg3 deficiency leads to a defect in the transition from pre–pro-B to early pro-B cells.

The deletion of Brg1 in HSCs also leads to defects in early pro-B cell development

Because the impairment of B cell development in Mx1-Srg3 fl/fl mice appeared to be due to a functional deficiency of the SWI/SNF-like BAF complex, it was likely that the inactivation of other subunits of the complex would also lead to similar defects in B cell development. To confirm this hypothesis, we generated Brg1-deficient chimeras using Brg1-shRNA (Fig. 5A, 5B). The deficiency in Brg1 also significantly decreased the frequency of B cells in the bone marrow (Fig. 5C). The frequencies of pre-B, immature B, and mature B cells were reduced in Brg1-deficient chimeras (Fig. 5D). Furthermore, most of the B cells were arrested at the stage of pre–pro-B cells, similarly to Srg3-deficient mice (Fig. 5E). These results indicate that proper activity of the SWI/SNF-like BAF complex is required for normal B cell development.

The SWI/SNF-like BAF complex is required for IL-7Rα expression and pro-B cell survival

Having shown that early pro-B cell development is blocked in the absence of Srg3/mBaf155, we speculated that pro-B cells of Mx1-Srg3 fl/fl mice would be defective in IL-7R signaling, which leads to more cell death and less proliferation (38, 39). In fact, we found that

**FIGURE 2.** B cell generation is impaired in the absence of Srg3/mBaf155. (A) Flow cytometric analysis of bone marrow B cells (B220+CD19+) from control and Mx1-Srg3 fl/fl mice. Numbers in plots indicate the percentage of populations. (B) Frequencies (left panel) and numbers (right panel) of bone marrow B cells (B220+CD19+) obtained from control and Mx1-Srg3 fl/fl mice. Each symbol represents one mouse; the lines show the mean percentage of bone marrow B cells. Error bars represent SEM (n = 12). (C) Flow cytometric analysis of splenic B220+CD9+ cells from control and Mx1-Srg3 fl/fl mice. Numbers indicate the percentage of splenic B cells. (D) Total cell numbers of splenic B cells. Each symbol represents one mouse; the lines show the mean value of splenic B cells (n = 7 for control and n = 9 for Mx1-Srg3 fl/fl mice). (E) Flow cytometric analysis of splenic B cells from control and Mx1-Srg3 fl/fl mice. B220+CD19+ cells were analyzed for the expression of surface molecules CD21 and CD23. Representative dot plot of FO (CD21+CD23+) and MZ (CD21–CD23+) B cells. Numbers indicate the percentage of indicated populations. (F) Frequency (left panel) and cell numbers (right panel) of MZ and FO B cells from control and Mx1-Srg3 fl/fl mice. Data represent average ± SEM (n = 6 for each group).
pro-B cells from Mx1-Srg3^{fl/fl} mice failed to upregulate IL-7Rα expression on the surface, especially at the early pro-B cell stage, whereas the control pro-B cells showed a high level of expression of IL-7Rα (Fig. 6A, 6B). In addition, we found that the Srg3-deficient pro-B cells showed a much higher frequency of Annexin V+ apoptotic cells compared with the control pro-B cells (Fig. 6C). These results indicate that pro-B cells lacking Srg3, especially early pro-B cells, are more susceptible to apoptosis, which may explain, at least in part, the severely reduced numbers of early pro-B cells in the bone marrow of Mx1-Srg3^{fl/fl} mice. Notably, pro-B cells purified from Mx1-Srg3^{fl/fl} mice showed higher levels of proapoptotic Bim expression and lower levels of antiapoptotic Bcl-xL expression (Fig. 6D).

To analyze the responsiveness of pro-B cells to IL-7 signaling, pro-B cells that were isolated from plpC-treated control or Mx1-Srg3^{fl/fl} mice were cultured on OP9 stromal cells with IL-7. Srg3-deficient pro-B cells were severely reduced on day 3, and fewer pro-B cells were observed on day 6 after culturing compared with control mice (Fig. 7A). In addition, Srg3-deficient pro-B cells showed a much higher frequency of Annexin V+ cells on day 3 of in vitro culture (Fig. 7B). Srg3-deficient pro-B cells also showed a significant reduction in cell proliferation compared with the control pro-B cells, as measured by BrdU incorporation after 3 d of in vitro culture of the pro-B cells (Fig. 7C). These results indicate that Srg3-deficient pro-B cells were defective in survival as a result of the impairment in IL-7 signaling.
Impaired B lineage-specific gene expression and VDJ rearrangement in Srg3/mBaf155-deficient pro-B cells

We next examined the expression of genes that are critical for early B cell development in pro-B cells isolated from pIpC-treated control and Mx1-Srg3 fl/fl mice. Srg3-deficient pro-B cells expressed much less E47 (Tcfe2a) and Ebf1, as well as their downstream target genes, such as Pax5, Il7ra, Cd79a, Cd79b, Igll1, Vpreb1, Rag1, and Rag2, than did the control pro-B cells (Fig. 8A, 8B). However, it is possible that the diminished expression of B lineage-specific genes in Srg3-deficient pro-B cells is due to fewer early pro-B cells. To rule out this possibility, we analyzed gene expressions in PD36 cells ectopically transduced with control or Srg3-shRNAs. We found that the expression of Ebf1 and its target genes was decreased in the absence of Srg3 (Fig. 8C). Brg1-deficient B cells also showed a reduction in the expression of B lineage-specific genes (Fig. 8D), which strongly indicated that Srg3-deficient pro-B cells fail to express genes that are crucial for B cell development.

In accordance with the reduced expression of Rag1 and Rag2 in Srg3-deficient CLPs and pro-B cells, the expression of the intracellular μ-H chain in Srg3-deficient pro-B cells was much lower than in the control pro-B cells (Fig. 8E). To determine
whether the Srg3-deficient pro-B cells failed to undergo V(D)J and DJ rearrangement of the IgH (Igh) genes, we analyzed the genomic DNAs isolated from pro-B cells obtained from control and Mx1-Srg3 fl/fl mice by semiquantitative PCR, followed by Southern blotting. The recombination frequencies of the proximal V H locus, including V H7183 and V HQ52, as well as the more distant V H locus, including V HGam3.8, V H3609 and V HJ558, were reduced in Srg3-deficient pro-B cells compared with control pro-B cells. Furthermore, the incidence of rearrangement of DH-JH was also much lower in Srg3-deficient pro-B cells than control pro-B cells (Fig. 8F). Consistent with the genomic DNA analysis, the rearranged transcripts of the proximal V H7183 gene and the distal V HJ558 gene were also decreased in Srg3-deficient pro-B cells compared with the control pro-B cells (Fig. 8G). These results indicate that the Srg3-deficient pro-B cells had defects in the V(D)J rearrangement of the Igh genes. This is consistent with impairment of IL-7R signaling, which is required for the regulation of V H gene rearrangement, in pro-B cells from Mx1-Srg3 fl/fl mice.

The core components of the SWI/SNF-like BAF complex directly bind to the promoter of Il7ra and Ebf1

Our results suggest that defects in the expression of B lineage-specific genes, including Ebf1 and IL-7Rα, on the surface of...
pro-B cells led to the impairment of pro-B cell development in Mx1-Srg3\(^{fl/fl}\) mice. We confirmed reduction of the Ebf1 transcript in PD36 cells transfected with a small interfering RNA duplex targeting Srg3 or Brg1 transcripts (Fig. 9A). It is known that the expression of Ebf1 is controlled through two other promoters (Fig. 9B): a distal promoter that is regulated by E2A and a proximal promoter that is regulated by PU.1 and Pax5 (40). Previous studies showed that PU.1 or Pax5 can cooperate with the BAF complex to activate target genes in various conditions (41–44). These results led us to investigate whether SRG3 directly regulates the expression of Ebf1 at the proximal region. To test the potential binding of SRG3, we used ChIP analysis with cross-linked chromatin extracted from PD36 cells. We found that immunoprecipitation with SRG3-specific Abs, but not isotype-control Abs, was enriched in the proximal promoter regions (Fig. 9C). BRG1 was also recruited to the same region (Fig. 9C).

In addition, ChIP analysis using primary pro-B cells showed the binding of SRG3 and BRG1 to the proximal promoter of the Ebf1 gene (Fig. 9D). The forced expression of Ebf1 in LSK cells purified from pIpC-treated Mx1-Srg3\(^{fl/fl}\) mice partially rescued the generation of B cells in vitro, whereas the generation of B cells was absent in Srg3-deficient LSKs that were ectopically transduced with control vectors (0.3937 ± 0.2889% versus 3.117 ± 0.5429% for B220+CD19+ cells) (Fig. 9E). However, ∼30% of the LSKs obtained from control mice differentiated into B cells (Fig. 9E). Although ectopic expression of Ebf1 produced B cell progenitors, the recovery of the cells was very inefficient in the absence of Srg3 (Fig. 9F). These results indicate that Ebf1 is very inefficient in activating B lineage-specific genes in the absence of Srg3 and suggest that it requires the SWI/SNF-like BAF complex for its full functional activity. We also found that SRG3 and BRG1 bind to the proximal promoter regions of Il7ra in PD36 cells and primary pro-B cells (Fig. 9G, 9H). These results collectively indicate that the SWI/SNF-like BAF complex is required for the transcriptional activation of Ebf1 and Il7ra in pro-B cells.

Discussion

The development of B lineage cells in the bone marrow occurs in a stepwise manner with critical checkpoints. The rearrangement of Ig genes and the proper expression of B lineage-specific genes are required for B cell commitment, survival, and proliferation. Epigenetic changes in the chromatin structure are essential for controlling the transcriptional regulation of these genes. In addition, the chromatin-remodeling complexes cooperate with sequence-specific transcription factors to promote the expression of several genes in many cell-specification events (17, 18). In this study, we demonstrated that Srg3/mBaf155, a core subunit of the SWI/SNF-like BAF complex, is essential for normal B cell development. Our data indicate that the BAF complex plays a pivotal role at both the CLP stage and the transition from pre-pro-B to early pro-B cells by regulating the expression of the B lineage-specific genes, such as Ebf1 and Il7ra.

Ebf1-deficient CLPs showed defects in the expression of B lineage-specific genes, although the numbers of CLPs are comparable between WT and Ebf1\(^{-/-}\) mice (37). E47-deficient mice showed severe reduction in AA4.1\(^{hi}\) CLPs (36). Notably, the
surface expression of AA4.1 on CLPs was significantly reduced in the absence of Srg3, whereas Flt3 expression remained largely unchanged. It was noted that AA4.1+ CLPs have the ability to give rise to B and T cells, but not myeloid lineage cells, both in vivo and in vitro (31). Consistent with the defects in the expression of AA4.1 on CLPs, Srg3-deficient CLPs displayed decreased expression of Ebf1 and its downstream target genes, such as Cd79a, Cd79b, Rag1, Rag2, and FoxO1, which are associated with early B cell development (31–34, 37). Although we cannot officially exclude the possibility that Srg3 directly regulates the expression of AA4.1, it is unlikely because there are still AA4.1+ med subsets in CLPs in Srg3-deficient mice. AA4.1+ pre-pro-B cells, but not AA4.1- cells, could develop into B220+CD43+ pro-B cells (7). Our data showed that Srg3-deficient pre-pro-B cells completely lack AA4.1+ population, although the number of pre-pro-B cells was normal in Srg3-deficient mice. Therefore, the pre-pro-B cells found in Srg3-deficient mice could not generate early pro-B cells, and these defects might be due to the impairment of the expression of B lineage-specific genes at the stage of CLPs in the absence of Srg3. Taken together, this appears to result in the severe reduction in the number of early pro-B cells in these mice.

Two major defects were observed in pro-B cells, consisting of pre-pro-B, early pro-B, and late pro-B cells, from Srg3-deficient mice. The first defect is the failure in cell survival and proliferation via IL-7 signaling. We have clearly shown that the Srg3-deficient pro-B cells, especially early pro-B stage cells, failed to express IL-7Ra on the surface, which resulted in more apoptosis and less proliferation of pro-B cells, even in the presence of IL-7. In addition, Srg3-deficient pro-B cells showed a higher expression of Bim and a significantly lower expression of Bcl-1XL relative to the control mice. These results are consistent with the decreased expression of IL-7Ra on the surface, which induces Bcl-1XL expression after the activation of Stat5 by Jak-mediated phosphorylation (45). Notably, the development of FO B cells was severely impaired by Srg3 deletion, whereas MZ B cells constituted a much higher proportion in the splenic B cell population in the absence of Srg3. These results are consistent with the phenotype in Il7ra-/- mice, in which the peripheral B cell defects are most apparent in the FO B cell compartment (46). Moreover, it was shown that the FO B cells increase in number, whereas the proportion of MZ B cells decreases in IL-7 transgenic mice (47).

The second defect in Srg3-deficient pro-B cells is the impaired expression of B lineage-specific genes, such as E47 (Tcfe2a), Ebf1, and Pax5. Our data also demonstrated that Srg3-deficient pro-B cells expressed less Rag1 and Rag2, which led to the failure of V(D)J rearrangement of the IgH genes and the expression of the cytoplasmic μ-chain. Moreover, the reduced expression of the downstream target genes of E2A and Ebf1, such as Il7ra, Cd79a, Cd79b, Igfl1, and Pax5, was observed in the absence of Srg3. Proper functioning of E2A or Ebf1 is required during B lineage differentiation. The loss of either E2A or Ebf1 results in a developmental block at the early stage of B cell development (12–14). The recombination of Dm-H1 segments, as well as the expression of B lineage-specific genes, is defective in the absence of either E2A or Ebf1. Unlike E2A-deficient mice, which show significantly lower numbers of CLPs and LMPPs (13), Ebf1-deficient mice have normal numbers of CLPs and pro-pre-B cell populations (14, 32). The overt characteristics of pro-B cell development blockade and impaired expression of B lineage-specific genes in CLPs in Srg3-deficient mice closely resemble those in Ebf1-/- mice. Therefore, a decreased expression of Ebf1 itself and/or downstream target genes is a plausible explanation for the compromised B lineage differentiation potential in lymphoid progenitors and the blockade of early B cell development in the absence of Srg3.

In agreement with the finding that the expression of Ebf1 and its downstream target genes was reduced in CLPs and pro-B cells obtained from Srg3-deficient mice, our results also provided evidence that the expression of Ebf1 was directly mediated by the SWI/SNF-like BAF complex. SRG3 and BRG1 were found to bind to the proximal promoter regions of the Ebf1 gene in pro-B cells. Although the relative activity of the distal promoter of Ebf1 (Ebf1-a) is higher than the activity of the proximal promoter (Ebf1-b) in the PD36 cell line, Ebf1-b transcript is predominantly expressed in all B lineage cells, especially in CLP and pro-B cells (the relative abundance of Ebf1-b mRNA is >100-fold higher than that of Ebf1-a) (40). Moreover, it was previously reported that recruitment of SWI/SNF-like BAF complex is required for the functional activity of Pax5, and the expression of Ebf1-b was reduced in Pax5-/- pro-B cells, whereas the expression of Ebf1-a was comparable (40–43). Therefore, it appears that the SWI/SNF-like BAF complex may act cooperatively with Pax5 to activate the expression of Ebf1 through the proximal promoter. Thus, in the absence of Srg3, Ebf1-b expression, which is reported to be transcribed by the proximal promoter, would be

FIGURE 7. Srg3-deficient pro-B cells are defective in survival in the presence of IL-7. (A) Flow cytometry of residual B220+ pro-B cells purified from pIpC-treated control or Mx1-Srg3fl/fl mice 6 d after in vitro culture with OP9 stromal cells. (B) Expression of Annexin V on pro-B cells from control and Mx1-Srg3fl/fl mice 3 d after in vitro culture (left panel). Numbers represent the percentage of Annexin V+ cells. Summary of the percentage of Annexin V+ cells from control and Mx1-Srg3fl/fl mice (right panel). Each symbol represents one mouse; the horizontal lines show the mean percentage of Annexin V+ cells (n = 6 for control and n = 5 for Mx1-Srg3fl/fl mice). (C) Flow cytometry of the proliferation of pro-B cells, using BrdU staining (left panel), purified from pIpC-treated control and Mx1-Srg3fl/fl mice by flow cytometry and then cultured for 3 d on OP9 stromal cells with IL-7. Numbers indicate the percentage of BrdU+ cells. Error bars indicate SEM of three independent experiments.
significantly decreased, and this will affect the early B cell development.

Enforced expression of Ebf1 in Srg3-deficient LSK cells rescued, although very inefficiently, the generation of B cells in vitro (GFP− control: 0.3937 ± 0.2889% versus GFP+Ebf1: 0.5429% for B220+CD19+ cells and control LSK: 28.57 ± 1.489%). Therefore, enforced expression of Ebf1 drives, with very low efficiency (~10% of control), the recovery of B cell progenitors that lack SWI/SNF-like BAF complex. This very low efficiency of the recovery appeared to be due to the absence of SWI/SNF activity in Srg3-deficient mice. Therefore, we believe that our results reflect the requirement of SWI/SNF for the functional activity of Ebf1, which is consistent with previous reports (41–43).

In summary, our results collectively establish the critical functions of the SWI/SNF-like BAF complex in the process of early priming of B lineage specification. The functional requirement for the BAF complex at both the CLP stage and the transition from pre–pro-B to early pro-B cells is correlated with defects in the expression of B lineage-specific genes, such as Ebf1, Il7ra, Tcfe2a,
and Pax5, and in the V(D)J rearrangement of the Igh locus in the absence of Srg3. Thus, our study suggests that proper control of epigenetic remodeling of chromatin is essential for the sequential steps of early B cell development.

**FIGURE 9.** The SWI/SNF-like BAF complex binds to the promoter regions of Ebf1 and Il7ra. (A) Semiquantitative PCR analysis of Ebf1 transcript level in PD36 cells transfected with small interfering RNA duplexes targeting GFP (si-GFP), Brg1 (si-Brg1), or Srg3 (si-Srg3). Data are representative of two independent experiments. Expression of gapdh served as a loading control. (B) Schematic representation of Ebf1 promoter regions and PCR primer sets for ChIP were depicted by arrows (P1–P4). (C) ChIP analysis of the binding of SRG3 and BRG1 to the Ebf1 proximal promoter locus on cross-linked chromatin from PD36 B cells, immunoprecipitated with anti-SRG3, anti-BRG1, or isotype control Ab (IgG1), followed by PCR amplification of input DNA or immunoprecipitated DNA with specific primers for the proximal promoter region. PCR product of gapdh intron region was used as a negative control. (D) ChIP analysis of cross-linked chromatin from B220⁺CD43⁺IgM⁻ pro-B cells, assessed as described in (C). PCR product of gapdh intron region served as a negative control, and immunoprecipitated chromatin with anti-H3K4 me3 was used as a transcriptional activation marker. Shown is a representative of two independent experiments. (E) Flow cytometric analysis of differentiation of B220⁺CD19⁺ B cells from control LSKs or Srg3-deficient LSK cells transduced with vectors expressing empty or Ebf1. Numbers indicate the percentage of B220⁺CD19⁺ B cells. (F) Summary of B220⁺CD19⁺ B cells differentiated from control LSKs or Srg3-deficient LSK cells transduced with vectors expressing empty or Ebf1. Data represent the average ± SEM (n = 3). (G) ChIP analysis of binding of SRG3 and BRG1 to the Il7ra promoter locus on cross-linked chromatin from PD36 B cells, immunoprecipitated with anti-SRG3, anti-BRG1, or isotype control Ab (IgG1), followed by PCR amplification of input DNA or immunoprecipitated DNA with specific primers for proximal promoter region. PCR product of gapdh intron region was used as a negative control, and enriched fragment immunoprecipitated with anti-PU.1 was used as a positive control. (H) ChIP analysis of cross-linked chromatin from B220⁺CD43⁺IgM⁻ pro-B cells, assessed as described in (F). Data are representative of two independent experiments. *p < 0.05, **p < 0.001.
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Disclosures
The authors have no financial conflicts of interest.

References