Zinc transporter SLC39A10/ZIP10 facilitates antiapoptotic signaling during early B-cell development

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The immune system is influenced by the vital zinc (Zn) status, and Zn deficiency triggers lymphopenia; however, the mechanisms underlying Zn-mediated lymphocyte maintenance remain elusive. Here we investigated ZIP10, a Zn transporter expressed in the early B-cell developmental process. Genetic ablation of *Zip10* in early B-cell stages resulted in significant reductions in B-cell populations, and the inducible deletion of *Zip10* in pro-B cells increased the caspase activity in parallel with a decrease in intracellular Zn levels. Similarly, the depletion of intracellular Zn by a chemical chelator resulted in spontaneous caspase activation leading to cell death. Collectively, these findings indicated that ZIP10-mediated Zn homeostasis is essential for early B-cell survival. Moreover, we found that ZIP10 expression was regulated by JAK-STAT pathways, and its expression was correlated with STAT activation in human B-cell lymphoma, indicating that the JAK-STAT-ZIP10-Zn signaling axis influences the B-cell homeostasis. Our results establish a role of ZIP10 in cell survival during early B-cell development, and underscore the importance of Zn homeostasis in immune system maintenance.

B-lymphocyte | apoptosis | cytokine | bone marrow | zinc-signaling axis

Zinc (Zn) has wide-ranging effects on immunity. Zn deficiency has uncovered the importance of Zn homeostasis in immune cell maintenance and function (1). Dramatic effects of Zn on immunity have been observed in several immune and allergy-related cells, including lymphocytes such as B cells (2–6). B cells develop in the bone marrow (BM); the initial commitment to pro-B cells is followed by their differentiation into pre-B cells, and subsequently into immature B cells, which express the B-cell receptor on their surface (7). The immature B cells reach the spleen as transitional B cells, further differentiating into follicular or marginal zone mature B cells (7). Although the perturbation of Zn homeostasis causes splenic atrophy associated with lymphocyte reduction, and compromises cellular and humoral immune responses (6), the mechanisms underlying how Zn controls immune cell function, and in particular, the impact on early B-cell development, have been largely unknown.

Zn homeostasis is tightly controlled by Zn transporter family members, Zrt- and Irt-like proteins (ZIPs, Zn importers) and zinc transporters (ZnTs, Zn exporters) (8), and recent studies revealed that alterations in Zn homeostasis mediated by specific Zn transporters play indispensable roles in a variety of cellular events (9). The intestinal Zn transporter ZIP4 is important for the initial absorption of dietary Zn, and patients with mutations in the *SLC39A4*/*ZIP4* gene suffer from the inherited disorder acrodermatitis enteropathica (10, 11). ZIP13 controls the formation of bone, teeth, and connective tissues by modulating BMP/TGF-β signaling (12), and its loss-of-function mutation causes spondylocheiro dysplastic Ehlers-Danlos syndrome in humans (12, 13). ZIP14 controls systemic growth by regulating G protein-coupled receptor (GPCR) signaling (14), and ZIP8 is involved in osteoarthritis (15) and negatively manipulates NF-κB activation (16). In addition, ZnT5 regulates cytokine production by controlling the activation of protein kinase C upon antigen exposure in mast cells (17). Thus, Zn homeostasis mediated by Zn transporters is linked to a wide variety of biological and regulatory functions, and the disruption of a Zn transporter-Zn axis can lead to various symptoms in the absence of redundant machinery (18).

Here we demonstrate a definitive role of ZIP10 in early B-cell development. We found that a loss of ZIP10 during an early B-cell stage specifically abrogated cell survival, resulting in the absence of mature B cells, which led to splenoatrophy and reduced Ig levels. The inducible deletion of *Zip10* in pro-B cells

Significance

Zinc deficiency is known to trigger lymphopenia, but the mechanisms behind zinc-mediated lymphocyte maintenance have been unclear. We demonstrated that zinc uptake into cells through the zinc transporter ZIP10 is essential for cell survival in early B-cell development. The ablation of ZIP10 caused an increase in caspase activity accompanied by reduced intracellular zinc in the early B-cell developmental stages. The JAK-STAT pathways regulated ZIP10 expression, and ZIP10 expression was correlated with STAT activation in B-cell lymphoma samples. Our results establish a physiological role for ZIP10 in early B-cell survival.

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increased the caspase activity because of the reduced intracellular Zn level, leading to cell death. This phenomenon was mimicked by the intracellular chelation of Zn. These findings indicated that Zn homeostasis via ZIP10 plays an indispensable role in early B-cell survival. We also demonstrated that the ZIP10 expression levels were regulated by STAT3/STAT5 activation, and that ZIP10 was highly expressed in human B-cell lymphoma samples in which both STAT proteins were activated, indicating that the JAK-STAT-ZIP10-Zn signaling axis is important for B-cell maintenance. Our results establish a functional link between ZIP10 and the survival of early stages of B cells, revealing a molecular mechanism underlying the requirement of Zn for maintenance of the immune system.

Results

Diminished Peripheral B Cells in *Zip10*$^{flox/flox}$*Mb1-Cre* Mice. It is well established that Zn deficiency causes severe lymphopenia, resulting in immune deficiency, which is mainly caused by a significant reduction in the developmental stages of B cells in the BM, leading to the depletion of antibody-producing mature B-cell populations (19); however, how Zn homeostasis helps to maintain early B-cell development has remained elusive. We noted that the *Slc39a10*/*Zip10* gene, whose encoded protein (ZIP10) was predicted to have multispan transmembrane domains, a relatively long extracellular sequence at the N terminus, and a long intracellular loop (Fig. S1 *A* and *B*), was expressed in a variety of tissues, including immune tissues, such as the thymus, spleen, and lymph nodes (Fig. S2*A*). ZIP10 was relatively highly expressed in cellular membrane of pro-B cells (Fig. S2*B*) on which it was glycosylated and formed oligomers (Figs. S1*C* and S3) (20, 21). Based on these findings but lacking evidence for immuno-physiological roles of ZIP10 in vivo, we first investigated whether ZIP10 plays a role in B-cell development by generating *Zip10*$^{flox/flox}$*Mb1-Cre* (*Zip10*^{Mb1}) mice, in which the *Mb1-Cre* transgene mediates constitutive Cre recombination in the B-cell line from the pro-B-cell stage (Fig. S4) (22). Although the *Zip10*^{Mb1} mice were healthy and grew normally, they displayed splenoatrophy (Fig. 1*A* and Fig. S5*A*), a reduced number of peripheral B cells (Fig. 1*B*), and decreased serum Ig levels (Fig. 1*C*), without significant changes in peripheral T-cell populations (Fig. S5 *B* and *C*). Further analysis showed that the reduction in peripheral B cells was attributed to a decrease in the B-cell progenitors (Fig. 2*A*), in which the ZIP10 expression was decreased accompanied by a reduction in the intracellular Zn level (Fig. 2 *B*–*E*). To confirm the intrinsic role of ZIP10 in B-cell development, we generated B cells in vitro from hematopoietic stem cells (HSCs) in the BM. Consistent with the in vivo data, a loss of ZIP10 led to impaired B-cell differentiation in vitro (Fig. 2*F*). We further confirmed the defective B lymphopoiesis in a coculture system of lineage-negative HSCs (Lineage$^-$/Sca-1$^+$/c-Kit$^+$, LSK-HSCs) with a stromal cell line, TSt-4 (Fig. 2 *G* and *H*) (23). These data demonstrated that ZIP10-mediated Zn homeostasis is critical for early B-cell development in a cell-autonomous manner.

ZIP10 Is Required for Early B-Cell Survival. To further clarify ZIP10's involvement in the early B-cell physiology, we generated another strain, *Zip10*$^{flox/flox}$*Rosa26ER*^{T2}*-Cre* (*Zip10*^{Rosa26}) mice (Fig. S4*A*), in which *Zip10* could be inductively down-regulated by 4-hydroxytamoxifen (4-OHT) treatment (24). We cultured pro-B cells from this strain with TSt-4 cells, followed by 4-OHT treatment (Fig. 3*A*). The inducible ablation of ZIP10 (Fig. 3*B*) reduced both the pro-B and pre–B-cell populations (Fig. 3*C*), accompanied by reduced intracellular Zn levels (Fig. 3*D*). Furthermore, the *Zip10*-deficient pro-B and pre-B cells underwent apoptosis, determined by annexin-V staining (Fig. 3*E*), with induced caspase-3 activation (Fig. 3*F*). These findings indicated that ZIP10 is required for the survival of early B-cell progenitors.

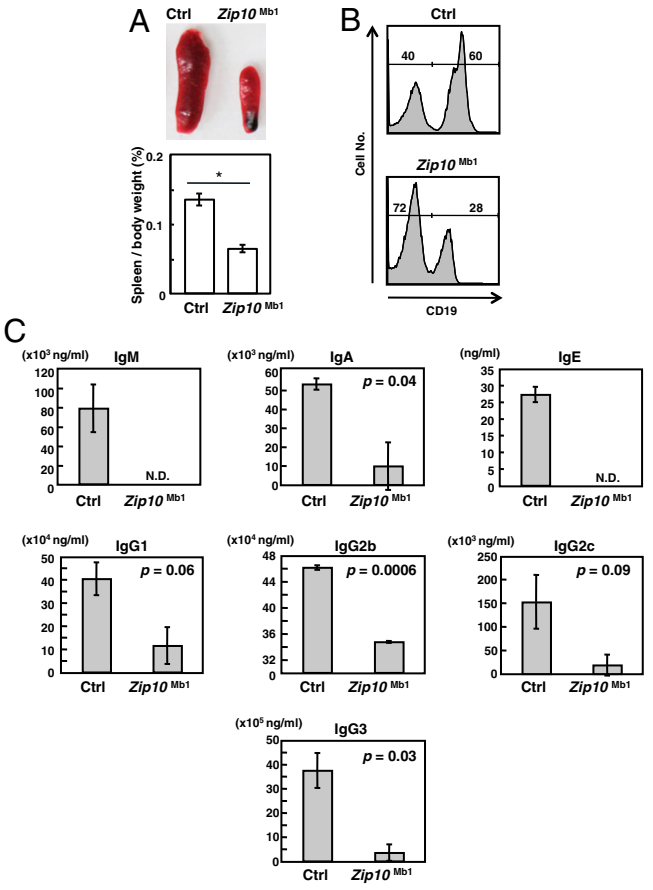

Fig. 1. *Zip10*^{Mb1} mice exhibit splenoatrophy with reduced numbers of peripheral B cells and diminished Ig levels. (*A*) Size (*Upper*) and ratio of tissue weight to body weight (*Lower*) of the spleen. Values represent means ± SD (n = 3 for each). *P < 0.05. (*B*) CD19$^+$ total B-cell population in spleen. (*C*) Serum Ig levels. Ctrl, control; *Zip10*^{Mb1}, *Zip10*$^{flox/flox}$*Mb1-Cre*; N.D., not detected. Values represent means ± SD (n = 2 for each).

Next, we investigated the molecular mechanism by which ZIP10 promotes B-cell survival using a cytokine-dependent BM-derived cell line, BAF-B03. The siRNA-mediated gene silencing of *Zip10* reduced its surface expression (Fig. 4*A*) accompanied by a decreased intracellular Zn level (Fig. 4*B* and Fig. S6*A*). In accordance with these observations, the *Zip10* RNA interference increased the apoptosis of the cells (Fig. 4*C*) and activated caspases involved in various apoptotic cascades: caspase-8 for Fas-FasL, caspase-9 for mitochondrial stress, caspase-12 for endoplasmic reticulum stress, and caspase-3 as an effector of apoptotic pathways (Fig. 4*D* and Fig. S6*B*) (25), suggesting that ZIP10-mediated Zn uptake exerts negative effects on caspase-dependent apoptotic pathways to maintain cell survival. To test this hypothesis, we examined the impact of intracellular Zn deprivation in the BAF-B03 cells. A selective Zn chelator, TPEN [*N,N,N′,N′*-Tetrakis(2-pyridylmethyl) ethylenediamine], induced apoptosis (Fig. 4*E*) with caspase activation (Fig. 4*F* and Fig. S7), and these effects were cancelled by Zn supplementation (Fig. 4 *E* and *G*), indicating that the Zn transported via ZIP10 is critical for the repression of caspase-mediated apoptosis.

Cytokine Signaling Controls ZIP10-Mediated Zn Homeostasis. Because the cytokine-mediated JAK-STAT signals are known to be important for early B-cell survival and development (26, 27), we next assessed whether cytokine signaling governs the ZIP10-mediated Zn homeostasis in early B-cell stages. We found that

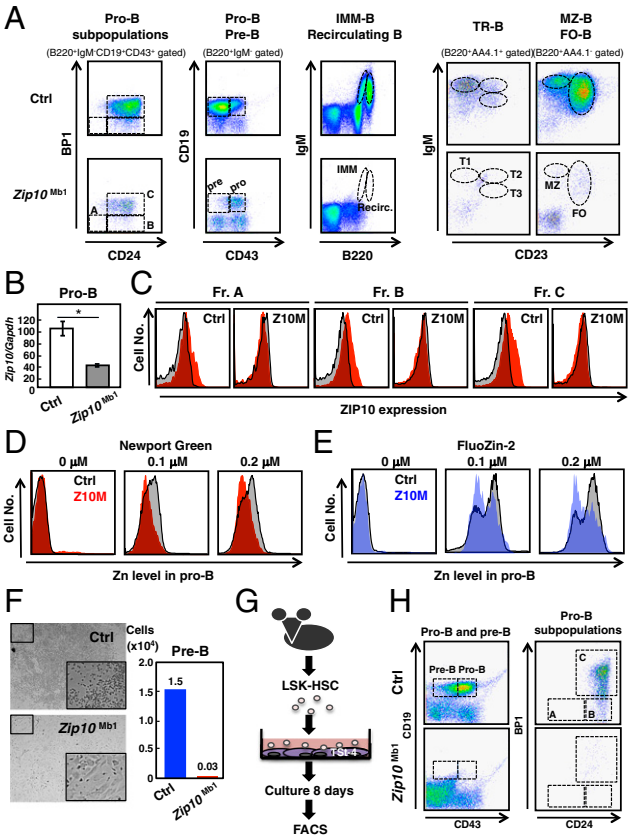

Fig. 2. ZIP10 controls B-cell development in a cell-intrinsic manner. (*A*) Populations of B-cell subsets in BM and spleen. IMM-B, immature B cells; TR-B, transitional B cells; MZ-B, marginal zone B cells; FO-B, follicular B cells. (*B*) *Zip10* mRNA expression in pro-B cells. Values represent means ± SD; $*P < 0.05$. (*C*) Surface ZIP10 protein expression in each pro–B-cell subpopulation in *A*. Red and gray histograms indicate ZIP10 levels detected with anti-ZIP10 antibody and isotype control, respectively. Ctrl, control; Z10M, *Zip10*^{Mb1} pro-B cells. (*D*) Intracellular Zn level in pro-B cells. Gray and red histograms indicate Newport Green intensities in the pro-B cells from control and *Zip10*^{Mb1} mice, respectively. (*E*) Intracellular Zn level in pro-B cells. Gray and blue histograms indicate FluoZin-2 intensities in the pro-B cells from control and *Zip10*^{Mb1} mice, respectively. (*F*) In vitro B-cell differentiation of BM cells. Whole BM cells were cultured in medium supplemented with 10% (vol/vol) FBS, 10 ng/mL recombinant mouse IL-7 and SCF for 14 d. (*Left*) Bright-field images of cultured cells. Magnified images of black-boxed area in the upper left show in the right corner. (*Right*) Number of cultured pre-B cells. (*G*) Schematic diagram of the coculture system using LSK-HSCs. LSK-HSCs were cultured on TSt-4 feeder cells in medium containing recombinant mouse IL-7 and SCF for 8 d, followed by FACS analysis. (*H*) LSK-HSCs were sorted from control or *Zip10*^{Mb1} mice, and were cocultured with TSt-4 cells. The pro-B, pre-B, and pro–B-cell subpopulations are shown.

stimulation with IL-7 plus stem-cell factor (SCF) or IL-7 alone induced *Zip10* expression in primary pro-B cells (Fig. 5*A*) and a pre–B-cell line, 2E8 (Fig. S8), respectively. Similarly, increases in surface ZIP10 protein expression and intracellular Zn level were observed in BAF-G133, a BAF-B03–derived cell line that expresses a chimeric receptor composed of the extracellular domain of the G-CSF receptor and intracellular domain of gp130 (Fig. 5*B*) (28). Notably, introduction of a dominant-negative form of STAT3 (Fig. 5*C*) or STAT5 siRNA (Fig. 5*D*) repressed the cytokine-induced ZIP10 expression, indicating that ZIP10-mediated Zn uptake depends at least partly on cytokine-JAK-STAT signaling. Finally, we identified two potential STAT-binding sites in the proximal regions of both the mouse and human *Zip10* promoters by TFSEARCH (Fig. S9), where activated-STAT3 and -STAT5 were bound (Fig. 5*E*). These results indicated that cytokine-mediated signals positively control the ZIP10 expression and Zn influx to regulate the B-cell survival in the early B-cell stages. In addition, we found that ZIP10 was highly expressed and colocalized with activated-STAT3 and -STAT5 in follicular B lymphoma cells in the salivary gland from human patients (Fig. 6 and Fig. S10*A*). Taken together, these findings suggest that ZIP10's expression is at least partly influenced by the activation status of STAT3 and STAT5 proteins, and that the JAK-STAT-ZIP10-Zn signaling axis has an important role in B-cell maintenance.

Discussion

In the present study, we demonstrated that ZIP10 promotes early B-cell survival by inhibiting the activation of caspases, and that ZIP10 expression and Zn homeostasis are regulated in a STAT-dependent manner. Thus, ZIP10-mediated Zn signaling is a novel regulator of early B-cell development, which establishes

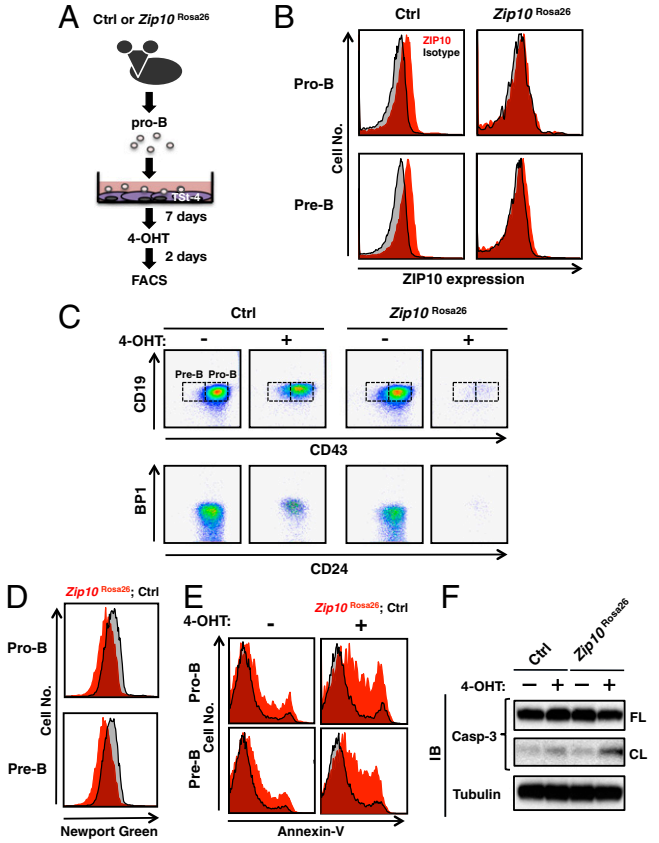

Fig. 3. Inducible deletion of *Zip10* triggers caspase activation leading to apoptosis. (*A*) Schematic diagram of the coculture system using pro-B cells in an inducible gene deletion. The pro-B cells were sorted and cocultured with TSt-4 cells for 7 d. The cells were then incubated with 0.05 μM 4-OHT for 2 d, followed by replacement with fresh medium and 2 more days of culture. The cells were collected and subjected to FACS analysis. (*B*) Surface ZIP10 protein expression in the pro-B and pre–B-cell populations after 4-OHT treatment. Red and gray histograms indicate ZIP10 levels detected by anti-ZIP10 antibody and isotype control, respectively. (*C*) Populations of pro-B and pre-B cells after the inducible deletion of *Zip10*. (*D*) Intracellular Zn level in pro-B and pre-B cells after the inducible deletion of *Zip10* shown in *A*. Gray and red histograms indicate Newport Green intensities in the pro-B and pre-B cells from control and *Zip10*^{Mb1} mice, respectively. (*E*) Annexin-V staining in pro-B and pre-B cells after the inducible deletion of *Zip10*. Gray and red histograms indicate annexin-V fluorescence levels in the pro-B and pre-B cells from control and *Zip10*^{Mb1} mice, respectively. (*F*) Caspase-3 (Casp-3) activation after the inducible deletion of *Zip10*. FL, full-length; CL, cleaved. Tubulin is shown as a loading control.

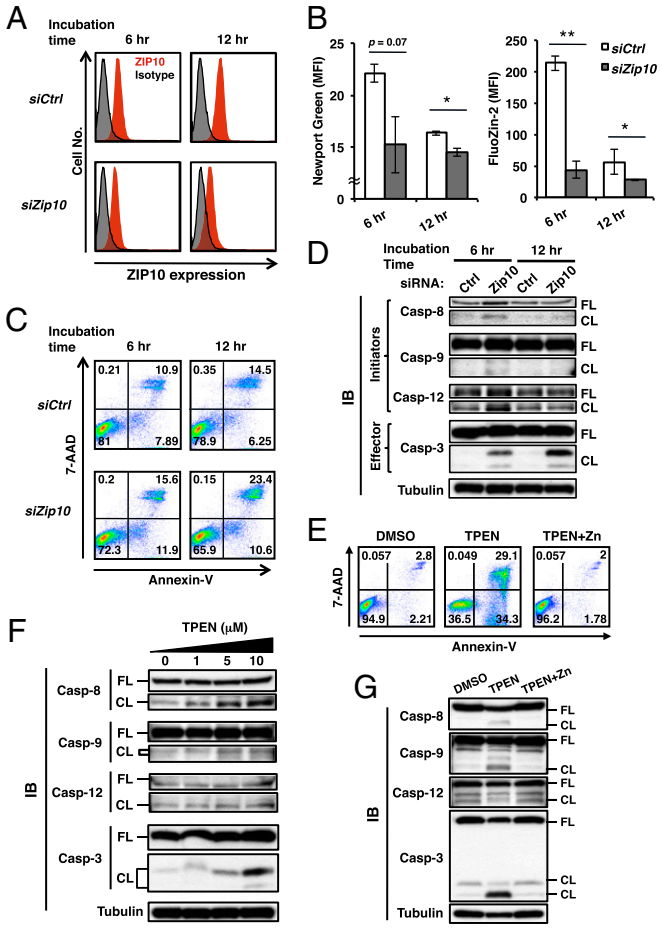

Fig. 4. ZIP10-mediated intracellular Zn status regulates caspase-dependent apoptotic pathways. (*A*) Surface ZIP10 protein expression in the *Zip10* siRNA-transfected mouse cytokine-dependent BM-derived BAF-B03 cells. *siZip10*, *Zip10* targeting siRNA; *siCtrl*, scrambled nontargeting siRNA. Red and gray histograms indicate ZIP10 levels detected by anti-ZIP10 antibody and isotype control, respectively. (*B*) Intracellular Zn level in *Zip10* siRNA-treated BAF-B03 cells. The mean fluorescent intensities (MFI) of Newport Green and FluoZin-2 are shown. Representative histograms are shown in Fig. S6*A*. $*P < 0.05$; $**P < 0.01$. (*C*) Annexin-V and 7-AAD staining in *Zip10* siRNA-treated BAF-B03 cells. The apoptotic cell populations (annexin-V$^+$7-AAD$^-$ and annexin-V$^+$7-AAD$^+$) are shown. (*D*) Activation of caspases in the *Zip10* siRNA-treated BAF-B03 cells. FL, full-length; CL, cleaved. Tubulin is shown as a loading control. (*E*) Annexin-V and 7-AAD staining in Zn-chelated BAF-B03 cells. Cells were treated with a Zn chelator, TPEN in the presence or absence of extracellular Zn. The apoptotic cell populations (annexin-V$^+$7-AAD$^-$ and annexin-V$^+$7-AAD$^+$) are shown. (*F*) Activation of caspases in Zn-chelated BAF-B03 cells. Indicated amounts of TPEN were added into culture medium. Tubulin is shown as a loading control. (*G*) Activation of caspases in Zn-chelated BAF-B03 cells in the presence or absence of extracellular Zn. Tubulin is shown as a loading control.

a functional link between Zn homeostasis and B-cell development (Fig. 7), and reveals the physiological significance of Zn in maintaining immune systems.

Since Zn deficiency was first described in the 1960s (29), numerous reports have emphasized Zn's physiological importance in the immune system. Aberrant Zn homeostasis leads to a regression of lymphoid tissue and compromises both B- and T-cell development (30). In particular, early B-cell development is severely affected by Zn deprivation in vivo (19). Acrodermatitis enteropathica patients, who have Zn deficiency because of loss of the intestinal Zn transporter ZIP4, display depressed immune functions accompanied by reduced lymphocyte numbers, resulting in death within a few years because of increased susceptibility to infections if left untreated (10, 11). We observed a similar mode of immunological disturbance in the present study. The ablation of ZIP10 caused decreased intracellular Zn levels, leading to a substantial reduction in the total B-cell populations (Figs. 1–3). Our results clearly demonstrated an essential role for cellular Zn homeostasis in B-cell development, providing a molecular basis for the immunodeficiency that results from Zn deficiency.

We showed that ZIP10 negatively regulates the activity of caspases by inducible gene knockout, RNA interference, and Zn chelation experiments (Figs. 3 and 4). Thus, ZIP10 is a novel survival factor for B-cell progenitors and has a suppressive effect on caspase activity through Zn uptake. Although in vitro studies have previously demonstrated that physiological concentrations of Zn modulate caspase activity (31–33), and that Zn's binding to

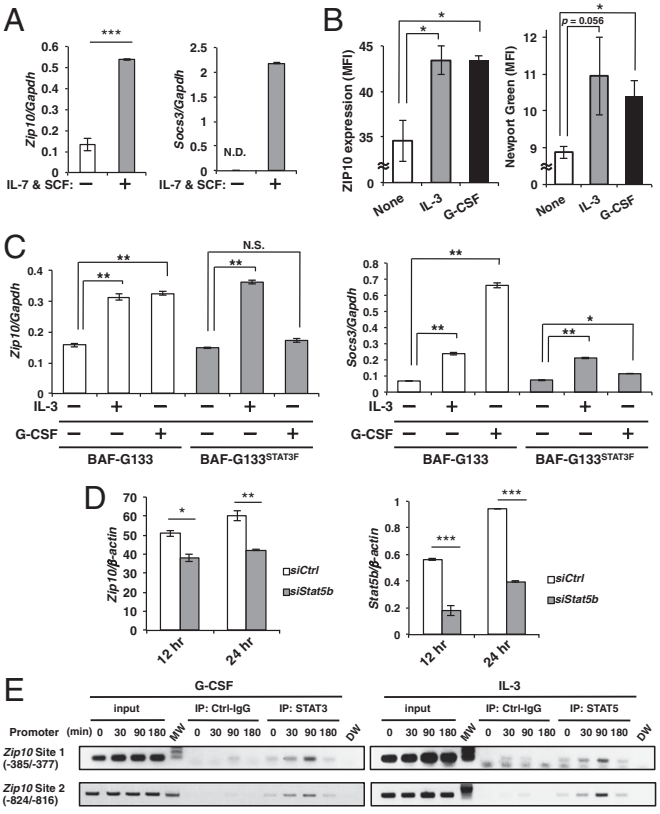

Fig. 5. JAK-STAT–mediated cytokine signaling regulates *Zip10* expression. (*A*) Pro-B cells were sorted from the BM of wild-type mice and stimulated with hematopoietic cytokines IL-7 and SCF for 3 h. (*Left*) *Zip10* mRNA expression; (*Right*) *Socs3* mRNA expression as a positive control. Values represent means ± SD. N.D., not detected. $***P < 0.001$. (*B*) Surface ZIP10 protein expression and intracellular Zn level in cytokine-treated BAF-G133 cells, which express a chimeric receptor consisting of the extracellular domain of the human G-CSF receptor and the intracellular domain of gp130 (28). BAF-G133 cells were stimulated with IL-3 or G-CSF for 12 h, followed by FACS analysis using anti-ZIP10 antibody and Newport Green. The MFI of ZIP10 and Newport Green are shown. $*P < 0.05$. (*C*) BAF-G133 and BAF-G133^{STAT3F} cells expressing a dominant-negative STAT3 were stimulated with IL-3 or G-CSF for 15 min. (*Left*) *Zip10* mRNA expression; (*Right*) *Socs3* mRNA expression as a positive control. Values represent means ± SD $*P < 0.05$, $**P < 0.01$; N.S, no significance. (*D*) *Zip10* mRNA expression in *Stat5b* siRNA-treated BAF-B03 cells. BAF-B03 cells were transfected with the indicated siRNA (*Stat5b*-targeting or nontargeting), then harvested after culturing for the indicated times. (*Left*) *Zip10* mRNA expression; (*Right*) *Stat5b* mRNA expression. Values represent means ± SD $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. (*E*) STAT3 and STAT5 binding to the proximal region of the *Zip10* promoter. BAF-G133 cells were stimulated with G-CSF or IL-3, and the binding of activated STATs to promoter regions of *Zip10* was analyzed by ChIP: (*Left*) STAT3, (*Right*) STAT5.

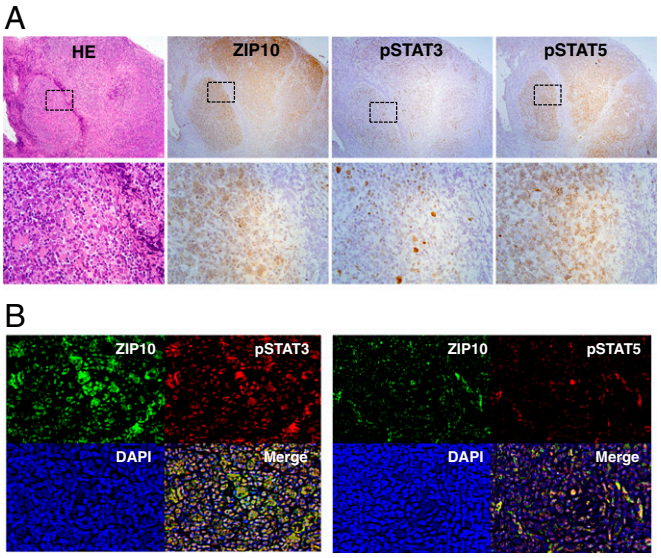

Fig. 6. Colocalization of ZIP10 and activated STAT3/STAT5 in human BCLs. (*A*) Histological analysis for the expression of ZIP10, activated STAT3 (pSTAT3) and STAT5 (pSTAT5) in human follicular lymphomas found in salivary glands. H&E staining and immunohistochemistry are shown. Other patient cases are shown in Fig. S10*A*. (*B*) Immunofluorescence analysis for the expression of ZIP10, activated STAT3 (pSTAT3) and STAT5 (pSTAT5) in human follicular lymphoma cells. (Magnification: *A*, *Upper*, 40×; *A*, *Lower*, 400×; *B*, 600 ×.)

cysteine residues in the catalytic domain of caspases inhibits their enzymatic activity (34), it was still elusive how Zn homeostasis modulates caspase-mediated apoptotic signaling in the physiological situation. It is well recognized that rigorous selection processes occur during B-cell maturation, in which down-regulation of the antiapoptotic factor B-cell lymphoma 2 (BCL2) induces apoptosis to prevent the generation and expansion of non- or self-reactive B-cell clones (19, 35). Our findings raise the possibility that Zn signaling mediated by ZIP10 exerts an antiapoptotic effect in coordination with BCL members for the fate decision of lymphocyte progenitors during their selection process.

There are several reports on the involvement of other Zn-sensitive signaling molecules in lymphopoiesis. Zn mediates the recruitment of the tyrosine kinase Lck to the T-cell receptor (TCR) signaling complex to facilitate TCR signaling (36, 37). Tyrosine phosphatase (38) and phosphodiesterase are also inhibited by Zn (14, 39, 40). Therefore, we do not exclude the possibility that the ZIP10-Zn signaling axis simultaneously modulates the related signaling pathways. A Zn finger transcriptional factor BCL6 is critical for cell survival at the pre–B-cell transitional stage (41). In addition, E2A is also essential for B-cell development, and its absence results in growth arrest (42) and caspase-dependent apoptosis (43). These findings suggest that the ZIP10-Zn signaling axis may also affect transcriptional control in early B-cell development. Intriguingly, our results indicated that the other cell-membrane localized ZIP family members expressed in B cells (Fig. S11) could not compensate for the loss of ZIP10 (Fig. S6*C*). In addition, forced Zn influx by pyrithione could not restore cell death induced by ZIP10 deficiency (Fig. S12), most likely because Zn plus pyrithione treatment triggers necrosis (44), suggesting that the ZIP10 regulates early B-cell survival through the tight regulation of Zn uptake in the specific Zn-signaling axis. In the future, more detailed investigations of the underlying molecular mechanisms, including the identification of ZIP10-binding proteins, which mediate between ZIP10-Zn and caspase activities, and exploration of the roles of intracellular or truncated ZIP10 (Fig. S3*C*) (as reported in ref. 20, for example), will help us understand how Zn signaling systems function with specificity.

It is of particular interest that activated STAT proteins regulated the ZIP10 expression upon cytokine stimulations (Fig. 5). Our findings indicated that cytokine (first signal) stimulation induces the activation of JAK-STAT proteins (second signal), which is then converted to a Zn signal (third signal) via ZIP10 (Fig. 7). A perturbation of these sequential signal conversions may profoundly affect immune function. Given that STAT signaling is an oncogenic consequence (45), that ZIP10 facilitates antiapoptotic effects in B-cell progenitors, and that ZIP10 is involved in the migration of some cell types (46), ZIP10 might play a role in human cancers and related diseases. This notion is consistent with the results of an in silico search, which showed high ZIP10 expression in various types of cancers including acute myeloid leukemia and acute lymphoid leukemia (Fig. S10*B*). In addition, the ZIP10 expression was highly correlated with STAT3/STAT5 activation in human follicular lymphoma and diffuse large BCL cells (Fig. 6 and Fig. S10*A*). Because most follicular lymphoma cells also overexpress BCL2 (47), the highly expressed ZIP10, at least in part, via JAK-STAT signaling may exacerbate malignancy by eliciting antiapoptotic effects in coordination with BCL2. STAT family members are activated not only by cytokines but also by growth factor receptors and oncogenic tyrosine kinases (45), so ZIP10 may be involved in various disease conditions associated with STAT activation.

Taken together, our results uncover an essential role of ZIP10 in early B-cell development, and reveal that ZIP10-mediated intracellular Zn homeostasis contributes to B-cell survival by inhibiting the activity of caspases. Notably, we also found that ZIP10 has an important role in the humoral immune response mediated by mature B cells (21), indicating that ZIP10 has unique functions in both early and mature B-cell populations by

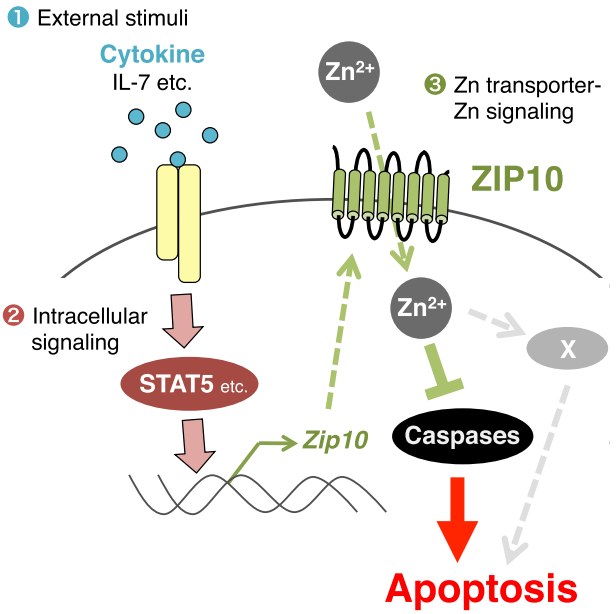

Fig. 7. Schematic model: ZIP10-mediated Zn signal promotes an antiapoptotic effect by inhibiting caspases in early B-cell developmental stages. Cytokine (first signal) stimulation induces JAK-STAT activation (second signal), and is subsequently converted to intracellular Zn signaling (third signal), through the up-regulation of Zn transporter ZIP10. This sequential intracellular "signal conversion" promotes early B-cell survival by inhibiting caspase activation or by an unknown mechanism via molecule X. A disruption in the signal conversion suppresses the survival signaling in B-cell progenitors, resulting in lymphopenia.

regulating different molecular and cellular events. Our studies on ZIP10 in B cells provide new insight into the relevance of Zn signaling in immuno-physiological events. Further exploration of the functions of ZIP10 will shed light on the relevance of Zn signaling in various aspects of the immune system.

Materials and Methods

Detailed descriptions of all of the materials and methods are provided in *SI Materials and Methods*, genotyping methods and primer sequences in Figs. S13–S15, and antibodies and reagents in Table S1.

Differences among multiple groups were compared by one-way ANOVA followed by a post hoc comparison using Fisher's protected least-significant difference test. The two-tailed Student *t* test was used to analyze differences between two groups.

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