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Signaling Pathway for the Development of Pre-B Cells

Tatsuaki Tsuruyama

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Abstract

Pre-B cells represent the immature stage of the B cell lineage and express genes for the pre-B cell receptor (preBCR). PreBCR consists of lambda 5 and VpreB and its expression elicits a rearrangement of the immunoglobulin heavy chain prior to rearrangement of the immunoglobulin light chain. The lambda 5 and VpreB form a surrogate light chain, which is a premature type of light chain immunoglobulin. PreBCR may cooperate or interact with the IL-7 receptor, which contributes to pre-B cell development. The preBCR distal signaling pathway recruits several adaptor proteins and protein kinases. This review aims to illustrate the framework of the signaling pathway that contributes to B cell lineage development and reconsiders the relationship between the preBCR and IL-7 receptors.

Keywords: pre-B cell receptor, IL-7 receptor, ZFP521, adaptor protein, rearrangement of immunoglobulin

1. Introduction

B cell lymphocytes develop from common lymphocyte progenitors (CLPs) to form mature B cells, plasma cells, or memory B cells in close association with immunoglobulin genes (*Ig*) rearrangement. *Ig* rearrangement (*IgR*) status is a phenotypic marker of immature B cell lineage. *IgR* occurs with the stepwise rearrangement of the V, D, and J segments of the immunoglobulin heavy chain (*IgH*) and immunoglobulin light chain (*IgL*) gene chain in the bone marrow. PreBCR is transiently expressed by developing precursor B cells.

The surrogate light chain is tentatively expressed on the membrane surface, followed by expression of mature *IgL*. Surrogate light and heavy chains form preBCR, involving the adaptor proteins CD79a and CD79b (alternatively termed as *Igα* and *Igβ*). It is not clear how preBCR reacts with external derived antigens and contributes to the formation of mature B cell receptors (BCR). To understand the role of preBCR, it is necessary to elucidate the preBCR distal signaling

pathway. It is well known that many key kinases participate in the BCR signaling pathway, such as spleen tyrosine kinase (Syk), bruton's tyrosine kinase (Btk), B lymphocyte kinase (Blk), and a transcriptional repressor Blimp (B lymphocyte-induced maturation protein). BP-1 (CD249) is another key player in the immature B cell lineage. In this article, the preBCR signaling pathway will be reviewed in reference to the BCR pathway in mice.

The B cell developmental stage has been extensively investigated using surface markers for the appropriate preBCR antibodies. The best known classification for immature phenotype markers is the Hardy's classification fraction (Fr) [1]. These markers range from Fr. A–D (Fr. A: B220+, IgM-, BP-1-, CD43+, and CD24-; Fr. B: B220+, IgM-, BP-1-, CD43+, and CD24+; Fr. C: B220+, IgM-, BP-1+, CD43+, and CD24+; Fr. C': B220+, IgM-, BP-1+, CD43+, and CD24^{high}; and Fr. D: B220+, IgM-, BP-1-, CD43-, and CD24+) with ongoing *IgH* recombination [2–4].

2. Signal cascade in pre-B cells

2.1. IL-7 and IL-7R signaling cascade

IL-7 and IL-7R play crucial roles in B cell development that function in parallel with *Ig* recombination. IL-7R is a heterodimer of the α chain (CD 127) and the common γ chain (γ_c chain). IL-7R α chains are primarily expressed in thymocytes, dendritic cells, mature T cells, and monocytes. This molecule is also expressed on the surface of pre-B cell lineages, but not on mature B cell lineages. In T cells and pre-B cells, the signal cascade via IL-7R recruits Janus kinases (JAKs), activates the signal transducer and activator of transcription (Stat) by phosphorylation. Among JAKs, Jak2 lacks Src homology binding domains (SH2/SH3) and JAK homology domains (JH1-JH7). The carboxy-terminal JH domain contains full kinase function (JH1), while the JH2 domain has significantly lower kinase activity than the JH1 domain. JAKs are recruited when IL-7 binds to IL-7R, leading to IL-7R auto-phosphorylation. Phosphorylated tyrosine residues of JAKs bind Stats with an SH2 domain [5] and Stat is phosphorylated by JAK. Phosphorylated STAT forms a dimer and translocate to the nucleus. Thereafter, the dimeric STAT binds to the promoter region containing palindromic gamma interferon activation site (GAS) elements that are positioned upstream of *Bcl-xL*, *Cyclin D1*, *pim1*, *c-myc*, and other genes inducing proliferation [6]. Suppressor of cytokine signaling (SOCS) controls this cascade in a feedback manner, because the *Socs* gene promoter has the GAS element. The SOCS family negatively controls by binding to JAKs. For example, the SOCS3 protein can bind to JAK2 kinase and inhibit its activity [7].

JAK2 mutations have been implicated in myeloid proliferative disorders such as chronic myeloid leukemia [8] polycythemia vera [9], primary myelofibrosis, essential thrombocythemia, and pre-B or lymphoblastic mature B ALL [10]. In these disorders, a change of valine to phenylalanine at the 617 position (V617F) in *JAK2* induces higher sensitivity to cytokines such as erythropoietin, thrombopoietin, and IL-7. In therapies targeting JAK2-STAT5 inhibition, *JAK2* mutations are observed in the poor prognostic *BCR-ABL1*-like subtypes of pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Mometinib and ruxolitinib were therapeutically effective for ALL. In the development of the lymphoblastic cells, the JAK-STAT pathway plays an important role [10].

IL-7R expression is also controlled in a developmental stage dependent manner. In parallel with *Ig* heavy chain rearrangement in pre-B cells (termed large pre-B or pre-B I), IL-7R expression augments and gradually decreases with expression of the surrogate light chain, consisting of VpreB and lambda5. Accordingly, the enhanced effect of the IL-7R signaling cascade is limited to the large pre-B or pre-B I stage. Accordingly, the pre-B ALL phenotype may depend on the IL-7R pathway. Thus, the activated pathway may determine the tumor phenotype. In the bone marrow, stromal cells actively produce IL-7 and promote the proliferation of bone marrow pre-B cells. Cytokine dependency is also observed in T cell leukemia/lymphoma and Human T-cell Leukemia/lymphoma Type 1 (HTLV-I)-induced adult T cell leukemia/lymphoma. When the pre-B cells develop immature-B cells, such dependency on the IL-7 and IL-7R pathway is lost and immature-B cells are recruited to extra-bone marrow area. In contrast, the excess IL-7R expression on the tumor progenitor cells may be activated by lower IL-7 in the peripheral environment, which may result in tumor development. However, the effect of IL-7 on human and mouse pre-B cells are different for proliferation and there have been reports that IL-7 may not be an essential factor in human cells.

Further, the relationship between IL-7R and Jak-STAT during *Ig* rearrangement remains controversial. In a previous study, MLV-integration based Stat5 activation was observed in pre-B cell lymphomas in an inbred strain of SL/Kh mice [9]. However, the dependency of proliferation on IL-7R is a part of lymphomagenesis, because the genetic background or microsatellite instability unique to this strain is also required [18]. For this reason, Stat5 activation is not sufficient for pre-B cell lymphoma or ALL [6].

2.2. IL-7R signaling cascade and *Ig* rearrangement

There has been discussion regarding the relationship between IL-7R signal cascades RAG1/2 (recombination activating gene 1/2) that mediate *Ig* rearrangement. In fact, it is not clear how transcription factors mediate transcriptional repression of Rag. Recombination activating implies that this gene is involved in immunoglobulin V-D-J recombination. The cleavage activity of RAG1 requires RAG2 as a partner. The RAG-1/2 complex nicks the Recombination Signal Sequence (RSS) that flanks the V, D, and J regions.

In the RAG1/RAG2 complex, RAG1 has the most catalytic activity while RAG2 provides a binding scaffold for the tight association with DNA [11]. There has been a controversial discussion in previous reports that the IL-7R pathway may enhance or suppress recombination. To explain the opposing effect of IL-7, it acts on *Ig* rearrangement in a dose-dependent manner. RSSs consists of three elements, heptamer sequences, spacer sequences, and nonamer sequences that flanks the V, D, and J sequences in the *IgH* and the V and J sequences in the *IgL* region. Spacer sequences are either 12 base pairs or 23 base pairs long and are located between the heptamer and nonamer sequences. Heptamer sequences are CACAGTG and nonamers are usually ACAAAAACC. After rearrangement, RSSs are spliced out of the final *Ig* mRNA. When B cells undergo *IgH* rearrangement, IL-7R expression increases, suggesting that IL-7R may be associated with the rearrangement. However, the downstream molecule Stat5 may contribute to suppression of the rearrangement. As a candidate for the suppression effect, Ebf1 expression is promoted by Stat5, suggesting a link between the negative regulations of Rag transcription [12].

Using model mice with Stat5 high expression, the *IgH* rearrangement was repressed. A schematic representation of immunoglobulin loci is illustrated below (Figures 1 and 2).

2.3. PreBCR and surrogate light chain

PreBCR is expressed on a large pre-B cell after or during *IgH* rearrangement. This contains a surrogate light chain consisting of VpreB and lambda 5. In addition, CD79a, CD79b, and the Ig muH chain participate in the formation of preBCR. Although it is not clear how antigens or ligands stimulate preBCR, signaling through preBCR controls allelic exclusion. One of the *IgH* locus rearrangements is inactive, promotes proliferation, and induces differentiation to small pre-B cells. *IgL* rearrangement follows this expression of preBCR. *IgL* rearrangement proceeds after the expression of preBCR in small pre-B cells. CD79a and CD79b contain tyrosine-rich ITAMs that can recruit the cytosolic SRC homology 2 (SH2)-domain-containing SYK following phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs). One of the pan-B cell markers, CD19 and CD45, interact with the preBCR to serve as regulators of positive signaling. In contrast, negative signaling and the regulatory mechanisms of preBCR have not been sufficiently addressed, Therefore, preBCR signaling has not been elucidated. It is possible that immunoreceptor tyrosine-based inhibitory motifs (ITIMs) function to recruit cytosolic

Figure 1. A scheme of B cell differentiation and immunoglobulin rearrangement.

Figure 2. Immunoglobulin gene loci. V_H, variable segment; D, diverse segment, J, junction segment. V_K, variable segment of kappa light chain.

protein tyrosine phosphatases (PTPs) such as SH2-domain-containing PTP 1 (SHP1). PIK3K is another adaptor protein of the CD79a and CD79b heterodimer. The RAS-ERK cascade and AKT-FOXO cascade may induce a positive signal inducing proliferation and BCL6, a marker of diffuse large B cell lymphoma/leukemia that may induce B cell lineage proliferation.

SYK can associate with other protein kinases, LYN and SLP65 /BLK or BLNK, that recruit BTK in a phosphorylation dependent manner. BTK associates with SLP-65, also known as BLNK or BASH, another important linker protein. BLNK suppresses Pre-B cell leukemogenesis through JAK3 inhibition [13–15]. BLNK KO mice developed pre-B cells or ALL in an experimental study. BLNK is part of a signaling complex involving Grb-2 and Vav, prior to arrangement of the cytoskeleton. BLNK, BTK, and PLCgamma 2 may form a complex that promotes IRF4 expression linked to *IgL* rearrangement that suppresses or downregulates the preBCR and IL-7R signal cascades simultaneously. In this way, the transition of *IgH* rearrangement to *IgL* rearrangement is controlled by the orchestration of various kinases and adaptor proteins. BLNK is involved in switching cell fate from proliferation to differentiation [16]. Additionally, BLNK recruits active H-Ras to the BCR complex, which is essential for sustained BCR surface expression and for the signal leading to functional ERK activation [17], potentially resulting in B-cell proliferation. Upregulation of *BLNK* may be a consequence of the negative-feedback mechanism and this upregulation results in tumor suppression. BLNK knockdown resulted in downregulation of *BP-1*, a pre-B cell marker [18]. Mice deficient in SLP65 /Blnk spontaneously develop pre-B cell leukemia [13], originating from pro-B cells with V(H)-to D-J(H) recombination. Nevertheless, *IgH* rearrangement was restricted to V(H)14-1 and V(H)14-2, V(H)14 *IgH* chains did not provide increased proliferative signals. PreBCR specificity did not contribute to oncogenic transformation.

Ikaros is required for the differentiation of large pre-B to small pre-B cells and is also required for the down-regulation of the preBCR, *Igκ* germline transcription, and *IgL* chain recombination. The Ikaros family are regulators of B-cell development by DNA-binding. Ikaros functions as a tumor suppressor in pre-B ALL [19] by controlling BCR-ABL1 kinase signaling from SRC kinase-activation to BLNK [20] or c-Myc expression [21]. Interestingly, BCR-ABL1 induces aberrant splicing of Ikaros in pre-B ALL [22]. The loss of Ikaros DNA-binding function leads to the progression of acute lymphoblastic leukemia [23]. Recently, MLL1 is found to be a regulator of preBCR signaling [24].

2.4. Orchestration of the preBCR and IL-7R signal cascades

In developing B cells, the IL-7R and preBCR synergize or act exclusively to induce proliferation [25]. However, preBCR is also critical to control differentiation through suppression of c-Myc function in large preB cells [26]. PreBCR is thus timely expressed in the transition of large to small preB cells [27]. PreBCR signaling does not affect interactions between the intronic enhancer and V (kappa) genes in proB cells. The kappa enhancers interact with the V (kappa) region already in proB cells. PreBCR signaling induces accessibility through functional redistribution of long-range chromatin interactions within the V(kappa) region [28]. *ZFP521* expression during cell growth is attenuated by the addition of IL-7 [16, 29]. Stimulation of preBCR modulated the growth of *ZFP521*-overexpressing cells [25]. IL-7 and preBCR control the development of preB cells into mature B cells [27, 30]. When IL-7R expression is gradually attenuated during the late stage of large preB cell development, preBCR signaling replaces the dominant pathway. B-cell

development is controlled in a stepwise manner where the first stage of development is completed before the subsequent stages are initiated.

There has been discussion as to whether preBCR functions as a tumor suppressor in the all cases of human acute lymphoblastic leukemia lymphoma (ALL). A distinct subset of human ALL is sensitive to preBCR [31]. The effects of preBCR stimulation were attenuated by the addition of IL-7 [16, 29]. Although both pathways are orchestrated during B-cell development [27], they are linked with immunoglobulin rearrangement [32]. During the development of pro-B cells into pre-B cells, IL-7 signaling is a major mediator with IL-7R expressed at high levels. In contrast, during the development of pre-B cells into mature B cells, preBCR signaling may be the dominant pathway after IL-7R expression is attenuated. Thereafter, mature BCR replaces preBCR. B-cell development is controlled in a stepwise manner in which the first stage of development is completed before the subsequent stages are initiated. In summary, the relationship between preBCR and the IL-7R cascade is complicated and forms an interactive network. The outcome of pre-B cell stimulation is difficult to predict in terms of proliferation or development. This network may be a necessary checkpoint for the developmental stage in a dose-dependent of IL-7 and other stimuli (**Figure 3**).

2.5. Other candidate cascades in pre-B cells

We previously reported that MLV insertion into the signal transducer and activator of transcription factor 5 (*Stat5*), Homeodomain-interacting protein kinase 2 (*Hipk2*), and Flt3-interacting zinc finger protein 1 (*Fiz1*) in the pre-B cell lymphoma genome. These genes encode proteins

Figure 3. The interactive responsiveness to stimulation through preBCR, IL-7, and other signaling cascades. In this scheme, bank 1 is selected for the modulator that is controlled by zfp521.

that are involved in pre-B cell-specific molecular signaling pathways such as IL-7R, CD43, and Fms-like tyrosine kinase 3 (FLT3). The dysregulation of this preBCR signal is responsible for pre-B cell lymphomagenesis [33]. ZFP521 contributed to mouse and human pre-B-cell lymphomagenesis (i.e., human B-cell lymphoblastic lymphoma). Pre-B cell proliferation depended on the activation of preBCR signaling molecules, which were upregulated by ZFP521.

2.6. Pre-B cell and acute lymphoblastic neoplasia in mouse

To date, pre-B cell lines from acute lymphoid tumors require strict cell culture conditions and consistent time course research. Using these cell line, IL-7 supply is critical, but IL-7 and v-Ha-ras expression are not individually sufficient to induce tumorigenicity. Their co-expression yields highly tumorigenic pre-B lymphoid cell lines [34].

On the other hand, as an experimental model, SL/Kh is known to be useful for tumorigenesis of pre-B cells and signaling pathways. This strain has two copies of AKV endogenous MLV and other retrovirus-derived fragments [3]. The expressed viral vector infects the host B cell progenitors and retroviral elements, such as promoters and enhancers promote *Stat5*, *c-myc*, *ZFP521*, *N-myc*, and other oncogenes [3, 6, 18, 35–37]. For this, this strain serves as an appropriate model for analysis of interaction between these molecules and their related signal pathways.

2.7. Role of ZFP521 during lymphoid differentiation

The mechanisms by which preBCR-related genes are controlled are not sufficiently understood relative to the mature B cell receptor. ZFP521 has been recently recognized as an important gene in pre-B cell lymphomagenesis. When ZFP521 is upregulated, BTK, BLNK, and BANK1 are involved in the preBCR signaling pathway and are comprehensively upregulated. ZFP521 contributes to the upregulation of *Ccnd3* and *Ccnd2*, enhancing the cell cycle and inducing proliferation. In a previous study, preBCR also activates the Ras-MEK-extracellular signal-regulated kinase (ERK) pathway, cell cycle exit, and light chain recombination by silencing *Ccnd3* [38]. *Ccnd3* gene expression is probably responsible for the growth of pre-B cells [39]. *BTK1* and *BANK1* are downstream of preBCR or BCR is controlled by ZFP521 and upregulation of *BTK1* and *BANK1* contributes to pre-B cell proliferation.

BANK1 is a modulator of the pre-B cell signaling pathway disrupted by IL-7R signaling that interacts with phospholipase gamma2 [40]. Overexpression of *BANK1* enhances BCR-induced calcium mobilization. Another lymphocytic associated kinase, LYN, associates with BANK1. LYN is activated with catalyst tyrosine phosphorylation of IP₃R (Inositol 1,4,5-trisphosphate receptor).

BTK is a useful diagnostic marker for Hodgkin's and B-cell non-Hodgkin's lymphoma [41]. BTK-dependent pathways are involved in maintaining the malignant phenotype in B-cell lymphomas and leukemias [31, 42–44]. Anti-apoptosis signaling in various B-cell malignancies requires BTK-dependent signals from the B-cell antigen receptor. A distinct subset of human ALL is selectively sensitive to preBCR antagonists, such as those employed for ibrutinib therapy for B cell malignancy [31, 42–44] In contrast, several reports suggested that BTK acts

as a tumor suppressor in the majority of human ALL cases [31, 41]. The BTK-dependent pathway is controlled in an expression dependent manner. Additionally, overexpressed BTK affects the survival or selection of B cells during the development of malignancies [45] and contributes to malignant transformation.

In humans, fusion of the *Pax5* exon 7 to *ZNF521* exon 4 has been observed in pre-B cell acute lymphocytic leukemia by genome-wide analysis of genetic alterations [46]. Importantly, this breakpoint is located near the conserved integration target sequence in human *ZNF521*, suggesting that the locus is active in pre-B cells as in *Pax5*, which is essential for pre-B cell development. Thus, we can conclude that aberrant release of the *ZFP521* gene control leads to pre-B cell lymphomagenesis through activation of pre-B cell-specific molecular signaling pathways. Moreover, c-Jun expression was observed in lymphoma tissues exhibiting *ZFP521* overexpression, suggesting that c-Jun is associated with lymphomagenesis [25].

MLV insertion into *ZFP521* gene was observed in the lymphomas with its insertion to *Sat5*, *Hipk2*, or *Fiz1* genes. These target tgenes may interact in the development of pre-B lymphoma. The dysregulation of this preBCR signal is responsible for pre-B cell lymphomagenesis [33]. *ZFP521* contributes to mouse and human pre-B cell lymphomagenesis (i.e., human B-cell lymphoblastic lymphoma). Pre-B cell proliferation depends on activation of preBCR signaling molecules including BANK1, which are upregulated by *ZFP521*.

ZFP521 is involved in tumorigenesis in pre-B cell lymphoblastic lymphoma through upregulation of preBCR signaling molecules, interfering with the IL-7R signaling pathway. *ZFP521* also mediates the expression of *Ccnd3*, *c-jun*, and other cell cycle-related genes. Therefore, these data suggested that *ZFP521* might be a promising target for targeted molecular therapy for ALL or B-precursor lymphoma.

3. Conclusion

The early stage of B cell differentiation is characterized by immunoglobulin rearrangement. Rearrangement is controlled by both the enzyme RAG 1/2, and IL-7R and preBCR signaling pathways. These two pathways sometimes function cooperatively, sometimes antagonistically and seem to support the timing of immunoglobulin gene rearrangement.

4. Materials

4.1. SL/Kh strain

SL/Kh is an inbred mouse strain that shares the AKV1 pro-virus with the AKR strain, which is susceptible to T cell leukemia/lymphoma [3]. This strain has been developed over 30 years through brother-sister mating and acquired susceptibility to MLV-mediated pre-B lymphoma [47]. AKV was mapped as the endogenous *ecotropic murine virus 11* (*Emv11*) onto chromosome 7 [48, 49]. In this strain, the pro- to pre-B cells expand in the bone marrow in a polyclonal manner before monoclonal expansion [18]. Afterwards, more than one copy of the proviral

genome is acquired during the development of lymphomagenesis. More than 90% of these mice spontaneously develop sIgM pro- or pre-B lymphomas, positive for preBCR, by 6 months of age. MLV genomes were integrated into Stat5, c-Myc, ZFP521, and other oncogenes in the lymphoma cell genome. Upregulation in expression of Stat5 and ZFP521 are not sufficient, as pre-B cell lymphomagenesis and a strain-dependent background are required [50, 51].

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Conflicts of interest

The authors have declared no conflict of interest.

Appendices and nomenclature

MLV	murine leukemia retrovirus
ZFP521	zinc finger protein 521
Stat5a	signal transducer and activator of transcription

Author details

Tatsuaki Tsuruyama^{1,2*}

*Address all correspondence to: tsuruyam@kuhp.kyoto-u.ac.jp

1 Department of Drug and Discovery Medicine, Pathology Division, Graduate School of Medicine, Kyoto University, Kyoto, Kyoto Prefecture, Japan

2 Clinical Research Center for Medical Equipment Development, Kyoto University Hospital, Kyoto, Japan

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