RESEARCH COMMUNICATION

Repression of *Flt3* by Pax5 is crucial for B-cell lineage commitment

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Early B-lymphopoiesis requires the growth-factor receptors, IL-7R and *Flt3*, and the activity of a number of transcription factors. One factor, Pax5, is required for commitment to the B-cell lineage, although the molecular mechanism by which this occurs is unknown. We demonstrate here that an important function of Pax5 is to repress *Flt3* transcription in B-cell progenitors, as Pax5-deficient pro-B cells express abundant *Flt3* that is rapidly silenced upon the reintroduction of Pax5, whereas enforced expression of *Flt3* in wild-type progenitors significantly impairs B-cell development. These findings demonstrate that the repression of *Flt3* by Pax5 is essential for normal B-lymphopoiesis.

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Hematopoietic stem cells (HSCs) have the dual capacity to self-renew and to give rise to cells of all the blood lineages. Although the identity and diversity of the intermediaries is still not completely understood, it is clear that an early differentiation step from the HSC gives rise to multipotent progenitors that have reduced capacity to self-renew. These progenitors up-regulate the tyrosine kinase receptor *Flt3* (also termed *flk2* and *CD135*) (Adolfsson et al. 2001) and efficiently reconstitute the lympho-myeloid but not the erythro-megakaryocytic lineages (Adolfsson et al. 2005). *Flt3* is later expressed on a subset of common myeloid progenitors, with dendritic cell (DC) potential (D'Amico and Wu 2003), and common lymphoid progenitors (CLPs) (Sitnicka et al. 2002).

Flt3^{-/-} mice have a severe deficiency in B-cell progenitors, and the mutant HSCs are impaired in their ability to reconstitute lymphoid and myeloid cells in recipient mice (Mackaretschian et al. 1995). Similarly, *Flt3L*-deficient mice have significantly reduced numbers of CLPs, B-cell progenitors, DCs, and natural killer (NK) cells (McKenna et al. 2000). *Flt3L* expressed by bone marrow stroma (Lisovsky et al. 1996), is a weak growth stimulator of *Flt3*⁺ CLPs in vitro and synergizes with stem cell factor (SCF) and IL-7 to promote the prolifera-

tion of lymphocytes and, most potently, DCs (Ray et al. 1996; Saunders et al. 1996; Veiby et al. 1996). The ability of *Flt3* to promote the expression of the IL-7 receptor on CLPs provides a mechanism by which this synergy occurs in early lymphopoiesis (Borge et al. 1999).

B-cell specification is controlled by the coordinate activity of a number of transcription factors, including E2A and EBF, which regulate rearrangement of the *IgH* locus and pre-B-cell receptor expression (for review, see Busslinger 2004; Singh et al. 2005). These factors also induce *Pax5*, which is essential for the restriction of lymphoid progenitors to the B-cell fate (Nutt et al. 1999). In the absence of Pax5, B lymphopoiesis in the bone marrow is blocked at the early pro-B (or pre-BI)-cell stage (Urbanek et al. 1994; Nutt et al. 1997). Analysis of *Pax5*^{-/-} pro-B cells has demonstrated that Pax5 plays a dual role in B-cell commitment, activating lineage-specific genes such as *CD19* and *mb-1* while repressing lineage inappropriate genes such as *M-CSF*, and *Notch1* (Nutt et al. 1998; Souabni et al. 2002). *Pax5*^{-/-} pro-B cells also have the capacity to self-renew indefinitely and to differentiate into multiple hematopoietic lineages (Nutt et al. 1999; Rolink et al. 1999). This similarity in developmental potential between *Pax5*^{-/-} pro-B cells and multipotent progenitors suggested that Pax5 normally represses genes necessary for the maintenance of the undifferentiated state. Here we show that one key function of Pax5 in B-lineage commitment is to directly repress *Flt3* expression, a process that is required for normal B-cell development.

Results and Discussion

Flt3 expression on pro-B cells

As Pax5 is essential for the commitment of progenitors to the B-cell fate, we hypothesized that Pax5 would directly repress genes associated with multipotency. *Flt3* represented an attractive candidate for Pax5 repression, as it is required for the repopulation capacity of multipotent cells (Adolfsson et al. 2001), and its expression is extinguished early in B-cell differentiation (Ogawa et al. 2000). Analysis of the c-kit⁺B220⁺ fraction (termed pro-B cells) isolated from the bone marrow of young mice (day 14) demonstrated only residual cell surface *Flt3*, whereas all *Pax5*^{-/-} pro-B cells expressed *Flt3* (Fig. 1A). *Flt3* expression could also be seen at the RNA level and was maintained in long-term *Pax5*^{-/-} pro-B-cell cultures (Fig. 1B,C). Thus *Flt3* expression is maintained in the absence of Pax5. To determine whether the ectopic *Flt3* is functional, we cultured *Pax5*^{-/-} pro-B cells for 3 d in a range of *Flt3L* concentrations. These experiments demonstrated a clear dose-dependent proliferation of *Pax5*^{-/-} pro-B cells in response to *Flt3L* (Fig. 1D).

Induction of Pax5 in pro-B cells represses *Flt3* expression

The continued expression of *Flt3* on *Pax5*^{-/-} pro-B cells suggested that one function of Pax5 in wild-type cells is to repress the *Flt3* gene. To test this hypothesis, we used a retroviral vector that expressed Pax5 fused to the ligand-binding domain of human estrogen receptor (*Pax5ER*) (Nutt et al. 1998). *CD19* is a direct transcrip-

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Figure 1. Pax5^{-/-} pro-B cells express Flt3. (A) FACS analysis of Flt3 on bone marrow c-kit⁺B220⁺ pro-B cells from 14-d-old wild-type (+/+) and Pax5^{-/-} (-/-) mice. c-kit⁺B220⁺ pro-B cells and established pro-B cell lines were analyzed for Flt3 expression by RT-PCR (B) and real-time PCR (C). Hprt was used to normalize cDNA input. (D) Pax5^{-/-} pro-B cells proliferate in response to Flt3L. Cells were cultured in IL-7 plus the indicated amounts of Flt3L for 72 h. The mean live cell number of triplicate wells $\pm$ SD is shown.

tional target that requires Pax5 for its expression (Nutt et al. 1998). As expected, Pax5^{-/-} pro-B cells transduced with Pax5ER were Flt3⁺CD19⁻, indicating that the fusion protein was inactive in the absence of ligand. Upon the addition of β -estradiol (E2), CD19 expression was strongly induced on the Pax5ER, but not control ER-expressing cells. Importantly cell-surface Flt3 levels significantly declined with Pax5ER induction (Fig. 2A). RT-PCR analysis of the kinetics of the repression indicated that Flt3 mRNA levels had dropped significantly within 4 h of Pax5 induction, indicating that this effect was likely to be direct (Fig. 2B,C).

To examine the timing of Flt3 repression during early B-cell differentiation, we have examined the c-kit⁺B220⁺ pro-B-cell compartment of wild-type mice. Interestingly, while the majority of pro-B cells were CD19⁺Flt3⁻, indicating Pax5 expression, a small fraction of Flt3⁺CD19⁻ and Flt3^{low}CD19⁺ cells was also present. This suggests that the progenitors progress from Flt3⁺CD19⁻ to Flt3^{low}CD19⁺ and Flt3⁻CD19⁺ stages with the onset of Pax5 expression, while in its absence, only Flt3⁺CD19⁻ cells were generated (Fig. 2D). This conclusion is supported by a recent study that described a B220⁺c-kit⁺CD19⁻NK1.1⁻ bone marrow cell population that can efficiently produce both lymphoid and myeloid cells in vitro (Balciunaite et al. 2005).

Pax5 binds to the Flt3 promoter

Pax5 regulates the expression of a number of genes by direct binding to their proximal promoter elements. As the Flt3 transcriptional start site and promoter region have not been reported previously, we used RNase protection assays to identify the Flt3 transcription initiation site. Analysis of the protected fragment placed the transcriptional start site at 47 nucleotides (nt) upstream of

the initiating methionine, a finding that was independently confirmed using 5' RACE (Supplementary Fig. 1).

The conservation of genomic sequences between species is generally indicative of functional importance. The putative Flt3 promoter sequence was aligned with the corresponding human, rat, and canine sequences. A region including the exon-1 and extending ~250 nt upstream of the transcriptional start site was highly conserved between these four species (Supplementary Fig. 1B). Beyond this, the sequences differed considerably. A search for transcription factor-binding sites in this region revealed two potential Pax5-binding sites (Supplementary Fig. 1B,C) (Czerny et al. 1993).

To determine whether Pax5 could bind to these sites within the Flt3 promoter, we performed electrophoretic mobility shift assays (EMSAs) using nuclear extracts and labeled oligonucleotides corresponding to either of the putative sites (Px1 and Px2). A high-affinity site from the human CD19 promoter served as a positive control (Fig. 3A; Kozmik et al. 1992). A DNA-binding complex was identified with each of the three probes that could be competed away with an unlabeled Pax5-binding site probe, but not with a fragment harboring a mutated Pax5

Figure 2. Flt3 is down-regulated by Pax5. (A) FACS analysis for CD19 and Flt3 on Pax5^{-/-} pro-B cell lines stably expressing an inducible Pax5ER or the estrogen receptor alone (ER) before and after 24-h treatment with 1 μ M E2. (B) RT-PCR analysis of CD19 and Flt3 regulation in the Pax5ER and ER cell lines at the indicated time points after E2 addition. (C) Real-time PCR analysis of Pax5ER induction time course. cDNAs were normalized using Hprt. (D) Analysis of CD19 and Flt3 levels on bone marrow pro-B cells from Pax5^{-/-} (-/-) and wild-type (+/+) mice.

Figure 3. Pax5 binds to the *Flt3* promoter. (A) EMSA of nuclear extracts (XT) from pro-B cell lines. The probes were 32 P-labeled oligonucleotides corresponding to a known Pax5-binding site from the *CD19* promoter (CD19) and the two putative sites in the *Flt3* promoter (Flt Px1 and Px2). Preincubation of nuclear extracts with either wild-type (wt) or a mutated (mut) CD19 oligonucleotide was used in competition assays (Comp). Pax5–DNA complexes were supershifted with a Pax5 antibody (Ab). Pax5-specific binding complexes (Px), nonspecific interactions (NS), complexes supershifted with Pax5 antibody (SS), or unbound probes (U) are indicated. (B) Determination of in vivo Pax5 binding to the *Flt3* and *CD19* promoter regions in pro-B cell lines using ChIP and real-time PCR analysis. Assay was performed $\pm$ Pax5 polyclonal antibody (Ab). Input is the equivalent amount of cross-linked chromatin prior to ChIP (the number in the column indicates the actual value for the input). DNA was quantified by real-time PCR using *Flt3* and *CD19* specific primers (see Supplemental Material) and calculated relative to *Pax5* $^{-/-}$ cells + Ab.

site (Fig. 3A). Preincubation of nuclear extracts with a Pax5-specific antibody resulted in a supershifted band, confirming that Pax5 bound to the sites identified in the *Flt3* promoter.

We used chromatin immunoprecipitation (ChIP) assays to investigate whether the Pax5 sites in the *Flt3* promoter are occupied in vivo. Pax5–chromatin complexes were precipitated from wild-type pro-B cells using a polyclonal antibody against Pax5, before PCR amplification with gene-specific primers. There was a fourfold enrichment of Pax5 binding to the known *CD19*-binding site in the sample immunoprecipitated with a Pax5 antibody compared with samples either from the *Pax5* $^{-/-}$ cells or in the absence of antibody (Fig. 3B). Most importantly, the *Flt3* promoter region spanning both Pax5-binding sites was similarly enriched in the sample derived from wild-type cells, providing compelling evidence that Pax5 bound to this region in vivo.

Enforced expression of *Flt3* on hematopoietic cells impairs B lymphopoiesis

To address the importance of *Flt3* down-regulation by Pax5 to B-cell development, we expressed *Flt3* throughout hematopoiesis using retroviral transduction. The MigR1 retroviral vector (Pear et al. 1998) was engineered to coexpress *Flt3* and GFP (MigR1–*Flt3*), allowing trans-

duced cells to be identified by flow cytometry. C57BL/6-Ly5.2 bone marrow stem cell cultures were transduced with the MigR1–*Flt3* or MigR1 constructs and injected into lethally irradiated C57BL/6-Ly5.1 congenic recipients. Reconstitution was assessed at 7 wk by analyzing Ly5.2 and GFP expression in hematopoietic organs (Fig. 4A).

Flt3 expression was readily detected on GFP hi cells from MigR1–*Flt3* HSC reconstituted mice in both bone marrow and thymus (Fig. 4A,B). However splenocytes showed few GFP hi cells, which did not express *Flt3*, suggesting *Flt3* negatively impacted on the ability of predominantly mature lymphocytes to repopulate the spleen. In contrast, cells transduced with the MigR1 vector, stably expressed GFP in all hematopoietic organs, and lacked endogenous *Flt3* (Fig. 4A,B).

A comparison of the percentages of total B cells (B220 $^{+}$ CD19 $^{+}$) in bone marrow GFP hi fractions from control and *Flt3*-expressing mice revealed a significant reduction in B cells in the presence of constitutive *Flt3* expression (Fig. 4C,D). B-cell developmental stages can be followed by the expression of surface markers, progressing from B220 $^{+}$ IL-7R $^{+}$ stage encompassing pro-B/pre-B cells to B220 $^{+}$ CD25 $^{+}$ pre-BII cells and finally to IgM $^{+}$ IgD $^{+}$ mature B cells. MigR1–*Flt3*-transduced stem cells gave rise to fewer B cells in each bone marrow compartment (Fig. 4C). The combination of reduced GFP $^{+}$ *Flt3* $^{+}$ cells in early B-cell differentiation and the selection against *Flt3* expression in peripheral B cells highlights the importance of the Pax5-mediated repression of *Flt3*.

Interestingly, within the non-B-cell compartment of the bone marrow, the majority of GFP hi cells were myeloid (Mac1 $^{+}$ Gr1 $^{+}$), while NK cells (NK1.1 $^{+}$ CD49b $^{+}$) were strongly reduced in numbers (Fig. 4C,D). In contrast, *Flt3* appeared to have no effect on thymocyte differentiation with similar numbers of CD4 $^{+}$ CD8 $^{+}$ cells compared with the MigR1 control, even though *Flt3* was readily detectable on CD4 $^{+}$ cells (Fig. 4B). Thus, the developing B- and NK-cell lineages appeared to be particularly susceptible to *Flt3* overexpression.

To determine whether ectopic *Flt3* reduced the frequency at which progenitors commit to the B-cell lineage or whether it inhibited maturing B cells directly, we examined the in vitro differentiation of B cells from stem cells transduced with the MigR1–*Flt3* or control viruses. Lineage-negative (Lin $^{-}$) GFP $^{+}$ cells were cultured for 7 d on OP9 stromal cells in B-cell conditions. Limiting dilution assays indicated that the MigR1-transduced cells very efficiently produced B220 $^{+}$ CD19 $^{+}$ B cells, whereas the MigR1–*Flt3*-transduced cells consistently produced clones at fourfold lower dilutions (Fig. 5A). A similar analysis of the cloning frequency of myeloid progenitors revealed no effect of *Flt3* expression (Fig. 5A). Importantly, proliferation assays performed on B220 $^{+}$ CD19 $^{+}$ committed B cells from the MigR1–*Flt3* cultures showed a similar dose-dependent response to *Flt3L* compared with that shown for *Pax5* $^{-/-}$ pro-B cells, indicating that the *Flt3*–*Flt3L* interaction did not inhibit, and actually promoted, the proliferation of committed B-cell progenitors (Fig. 5B). Taken together, these data indicated that the reduced B-lymphopoiesis in *Flt3* overexpressing mice resulted from lower B-cell precursor frequency and not a pronounced effect of *Flt3* on committed B cells.

The data presented here demonstrate that the repression of *Flt3* by Pax5 is an essential step in the process of

Figure 4. Enforced *Flt3* expression impairs B-cell development. (A) C57BL/6-Ly5.2 bone marrow stem cell cultures were transduced with GFP expressing MigR1 or MigR1-Flt3 retroviral vectors and used to reconstitute lethally irradiated C57BL/6-Ly5.1 recipients. Flt3 expression in donor Ly5.2⁺ GFP^{lo} (gray box) and GFP^{hi} (black box) cells was determined after 7 wk by FACS. (B) FACS analysis of Flt3 GFP^{hi} cells. (C) GFP^{hi} cells in the bone marrow compartment were identified using the indicated markers. Data are representative of five experiments. (D) Percentage of bone marrow B cells (B220⁺CD19⁺), myeloid cells (Gr1⁺Mac1⁺), NK cells (NK1.1⁺CD49b⁺), and thymocytes (CD4⁺CD8⁺) derived from GFP^{hi} cells. Mean of five experiments $\pm$ SEM is shown. (*) $P < 0.05$ paired Student's *t*-test.

B-cell lineage commitment. Initial studies of Flt3- and Flt3L-deficient mice reported reduced frequencies of B-cell progenitors, suggesting that Flt3 signaling was important for early B cells (Mackarehtschian et al. 1995; McKenna et al. 2000). Later studies, however, showed that the reduced B-lymphopoiesis in these strains reflects the requirement of the Flt3-Flt3L interaction for the maintenance of normal CLP numbers (Adolfsson et al. 2001; Sitnicka et al. 2002). These data support our expression analysis to indicate that Flt3 is specifically important at the CLP stage, before the induction of *Pax5*. As well as displaying synergy with cytokines such as IL-7 to support the survival and/or proliferation of lymphocytes, Flt3L also induces DC differentiation (Saunders et al. 1996), demonstrating that its role in early progenitors affects all CLP-derived lineages. The reduced B- and NK-cell differentiation, but normal thymopoiesis, we observed after ectopic expression of *Flt3* suggests the inhibitory function of prolonged Flt3 occurs after the CLP stage of differentiation. Moreover, as our in vitro assays showed committed B220⁺CD19⁺ cells ectopically expressing *Flt3* did not appear impaired in their proliferative response, we propose that the inhibitory effect of continued *Flt3* expression occurs at the transition between the CLP and the committed B-cell progenitor. We postulate that enforced Flt3 in this population allows these multipotent progenitors to be influenced by other stimuli, such as those favoring DC or T-cell differentiation, thereby impairing the capacity of CLP to produce the normal complement of B-cell precursors.

The transcriptional regulation of the commitment to the B-cell lineage has been a subject of intense interest in recent years, and a model has emerged where factors including Ikaros and PU.1 are required for the specification of the CLP (Fig. 5C; for review, see Busslinger 2004; Singh et al. 2005). These proteins induce the expression of two essential growth factor receptors, *IL-7R α* and *Flt3*, in lymphoid progenitors (Nichogiannopoulou et al. 1999; DeKoter et al. 2002). Once these progenitors are specified, EBF and E2A are required for the initiation of the B-cell expression program. An important consequence of E2A-EBF activity is the expression of *Pax5* (O'Riordan and Grosschedl 1999), which mediates exclusive B-cell commitment by coordinately activating B-cell-specific

Figure 5. *Flt3* impairs pro-B cell formation in vitro. (A) Cloning frequency of B and myeloid lineage progenitors transduced with MigR1 or MigR1-*Flt3*. Data from the same experiment are linked by a line. (B) GFP⁺ pro-B cells were cultured in IL-7 plus the indicated amounts of Flt3L for 72 h. The mean live cell number of triplicate wells $\pm$ SD is shown. (C) Multistep model of B-cell lineage commitment. Key transcriptional regulators and cell surface molecules are shown.

transcripts, such as *CD19*, and repressing genes important for the adoption of alternative cell fates, such as *MCSF-R* (for macrophages) and *Notch1* (for T cells) (Nutt et al. 1999; Souabni et al. 2002). While this model satisfactorily explains how Pax5 represses the ability to differentiate along the macrophage or T-cell pathway, it has not revealed how the E2A-EBF-Pax5 axis results in the loss of the more immature multipotent state. We now propose an extension to the lineage commitment model where in early B-cell differentiation, Pax5 represses a crucial growth factor receptor, *Flt3*, which is required for the multipotency of early lymphoid progenitors, and thereby facilitates unilineage differentiation by a negative feedback mechanism (Fig. 5C).

Materials and methods

Mice

Pax5^{-/-} (Urbanek et al. 1994) mice were maintained on a C57BL/6 background and genotyped as described (Nutt et al. 1997).

Flow cytometry

The mAbs against CD4, CD8, Ly5.2, Ter119, NK1.1, Mac1, Gr1, CD19, B220, IgM, and Flt3 were purified and conjugated in our laboratory. Labeled mAbs against CD25, IL-7R, TCR β , c-kit, CD49b, and IgD were from BD Biosciences. For flow cytometry, single-cell suspensions were stained with the appropriate mAb in PBS containing 2% FCS. Biotinylated mAbs were revealed by Cy5- or PE-streptavidin (Southern Biotech). Cells were analyzed on a LSR (BD Biosciences) and sorted using a FACS DIVA (BD Biosciences).

Cell culture

Pro-B-cell lines were derived and propagated as described (Nutt et al. 1997). Recombinant Flt3L was added to some cultures. The Pax5ER fusion protein and control ER retroviral vectors were introduced into *Pax5*^{-/-} pro-B cells and activated by 1 μ M E2 as described (Nutt et al. 1998). Bone marrow progenitors were cultured in HSC media (DMEM, 15% FCS, 10% IL-6 supernatant, 100 ng/mL SCF, 120 ng/mL Flt3L, 50 ng/mL TPO, 1 mM L-glutamine, 50 μ M 2-mercaptoethanol). OP9 stromal cells were cultured as described (Schmitt et al. 2004).

RT-PCR and real-time PCR

Total RNA was isolated from cells using Trizol (Invitrogen) and was reverse-transcribed with M-MLV RT (Promega) according to the manufacturer's instructions. Quantitative real-time RT-PCR was performed using SYBR Green PCR (Qiagen) on an ABI PRISM 7900HT cyclor (ABI). cDNA input was normalized to *Hprt*. Primers are in the Supplemental Material.

EMSA

Nuclear extracts (Andrews and Faller 1991) and EMSAs were performed as described (Barberis et al. 1990). End-labeled double-stranded oligonucleotides containing a Pax5-binding site from human *CD19* (Kozmik et al. 1992), *Flt3* P_{x1}, and P_{x2} were assayed. Sequences are in the Supplemental Material. A mutated *CD19* site was used as a nonspecific competitor and complexes supershifted by incubation with a Pax5 mAb made in our laboratory.

ChIP assay

ChIPs were performed as described previously with minor modifications (Forsberg et al. 2000). A Pax5 polyclonal antibody (2 μ g, sc-1974; Santa Cruz) was immunoprecipitated with Protein G-Sepharose (100 μ L) pre-blocked with dIdC (10 μ g), BSA (10 μ g), and sheared salmon sperm DNA (10 μ g). PCR was performed using the SYBR Green PCR kit on an ABI PRISM 7900HT cyclor. Products were amplified with similar efficiency, and absolute quantitation was performed using a standard curve. Primer sequences are in the Supplemental Material.

Retroviral expression of *Flt3* in bone marrow stem cells

The mouse *Flt3* cDNA was cloned into the MigR1 vector upstream of an IRES-GFP cassette (Pear et al. 1998). See Supplemental Material for a detailed description of the protocol for retroviral transduction and stem cell transplantation. For B-cell and myeloid differentiation studies, transduced progenitors were sorted as GFP⁺Lin⁻ (Gr1⁻CD19⁻NK1.1⁻Ter119⁻TCR β ⁻) and cocultured on OP9 (+1% IL7/Flt3L [5 ng/mL], B cells) or ST2 (+SCF [100 ng/mL], myeloid cells) stromal cells for 7 d. Progenitor frequencies were determined by limiting dilution assays. Proliferation assays with the Flt3⁺B220⁺CD19⁺ cells differentiated in vitro were performed in OP9 B-cell media containing IL-7 and a titration of Flt3L (0–200 ng/mL). Total live cell number was determined by FACS.

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References

- Adolfsson, J., Borge, O.J., Bryder, D., Theilgaard-Monch, K., Astrand-Grundstrom, I., Sitnicka, E., Sasaki, Y., and Jacobsen, S.E. 2001. Up-regulation of Flt3 expression within the bone marrow Lin-Sca1+c-kit⁺ stem cell compartment is accompanied by loss of self-renewal capacity. *Immunity* 15: 659–669.
- Adolfsson, J., Mansson, R., Buza-Vidas, N., Hultquist, A., Liuba, K., Jensen, C.T., Bryder, D., Yang, L., Borge, O.J., Thoren, L.A., et al. 2005. Identification of Flt3⁺ lympho-myeloid stem cells lacking erythro-megakaryocytic potential: A revised road map for adult blood lineage commitment. *Cell* 121: 295–306.
- Andrews, N.C. and Faller, D.V. 1991. A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. *Nucleic Acids Res.* 19: 2499.
- Balcunaite, G., Ceredig, R., Massa, S., and Rolink, A.G. 2005. A B220⁺CD117⁺CD19⁻ hematopoietic progenitor with potent lymphoid and myeloid developmental potential. *Eur. J. Immunol.* 35: 2019–2030.
- Barberis, A., Widenhorn, K., Vitelli, L., and Busslinger, M. 1990. A novel B-cell lineage-specific transcription factor present at early but not late stages of differentiation. *Genes & Dev.* 4: 849–859.
- Borge, O.J., Adolfsson, J., and Jacobsen, A.M. 1999. Lymphoid-restricted development from multipotent candidate murine stem cells: Distinct

- and complimentary functions of the c-kit and flt3-ligands. *Blood* **94**: 3781–3790.
- Busslinger, M. 2004. Transcriptional control of early B cell development. *Annu. Rev. Immunol.* **22**: 55–79.
- Czerny, T., Schaffner, G., and Busslinger, M. 1993. DNA sequence recognition by Pax proteins: Bipartite structure of the paired domain and its binding site. *Genes & Dev.* **7**: 2048–2061.
- D'Amico, A. and Wu, L. 2003. The early progenitors of mouse dendritic cells and plasmacytoid predendritic cells are within the bone marrow hemopoietic precursors expressing Flt3. *J. Exp. Med.* **198**: 293–303.
- DeKoter, R.P., Lee, H.J., and Singh, H. 2002. PU.1 regulates expression of the interleukin-7 receptor in lymphoid progenitors. *Immunity* **16**: 297–309.
- Forsberg, E.C., Downs, K.M., Christensen, H.M., Im, H., Nuzzi, P.A., and Bresnick, E.H. 2000. Developmentally dynamic histone acetylation pattern of a tissue-specific chromatin domain. *Proc. Natl. Acad. Sci.* **97**: 14494–14499.
- Kozmik, Z., Wang, S., Dorfler, P., Adams, B., and Busslinger, M. 1992. The promoter of the CD19 gene is a target for the B-cell-specific transcription factor BSAP. *Mol. Cell. Biol.* **12**: 2662–2672.
- Lisovsky, M., Braun, S.E., Ge, Y., Takahira, H., Lu, L., Savchenko, V.G., Lyman, S.D., and Broxmeyer, H.E. 1996. Flt3-ligand production by human bone marrow stromal cells. *Leukemia* **10**: 1012–1018.
- Mackarechtschian, K., Hardin, J.D., Moore, K.A., Boast, S., Goff, S.P., and Lemischka, I.R. 1995. Targeted disruption of the flk2/flt3 gene leads to deficiencies in primitive hematopoietic progenitors. *Immunity* **3**: 147–161.
- McKenna, H.J., Stocking, K.L., Miller, R.E., Brasel, K., De Smedt, T., Maraskovsky, E., Maliszewski, C.R., Lynch, D.H., Smith, J., Pulendran, B., et al. 2000. Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. *Blood* **95**: 3489–3497.
- Nichogiannopoulou, A., Trevisan, M., Neben, S., Friedrich, C., and Georgopoulos, K. 1999. Defects in hemopoietic stem cell activity in Ikaros mutant mice. *J. Exp. Med.* **190**: 1201–1214.
- Nutt, S.L., Urbanek, P., Rolink, A., and Busslinger, M. 1997. Essential functions of Pax5 (BSAP) in pro-B cell development: Difference between fetal and adult B lymphopoiesis and reduced V-to-DJ recombination at the IgH locus. *Genes & Dev.* **11**: 476–491.
- Nutt, S.L., Morrison, A.M., Dorfler, P., Rolink, A., and Busslinger, M. 1998. Identification of BSAP (Pax-5) target genes in early B-cell development by loss- and gain-of-function experiments. *EMBO J.* **17**: 2319–2333.
- Nutt, S.L., Heavey, B., Rolink, A.G., and Busslinger, M. 1999. Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. *Nature* **401**: 556–562.
- Ogawa, M., ten Boekel, E., and Melchers, F. 2000. Identification of CD19–B220+c-Kit+Flt3/Flk-2+ cells as early B lymphoid precursors before pre-B-I cells in juvenile mouse bone marrow. *Int. Immunol.* **12**: 313–324.
- O'Riordan, M. and Grosschedl, R. 1999. Coordinate regulation of B cell differentiation by the transcription factors EBF and E2A. *Immunity* **11**: 21–31.
- Pear, W.S., Miller, J.P., Xu, L., Pui, J.C., Soffer, B., Quackenbush, R.C., Pendergast, A.M., Bronson, R., Aster, J.C., Scott, M.L., et al. 1998. Efficient and rapid induction of a chronic myelogenous leukemia-like myeloproliferative disease in mice receiving P210 bcr/abl-transduced bone marrow. *Blood* **92**: 3780–3792.
- Ray, R.J., Paige, C.J., Furlonger, C., Lyman, S.D., and Rottapel, R. 1996. Flt3 ligand supports the differentiation of early B cell progenitors in the presence of interleukin-11 and interleukin-7. *Eur. J. Immunol.* **26**: 1504–1510.
- Rolink, A.G., Nutt, S.L., Melchers, F., and Busslinger, M. 1999. Long-term in vivo reconstitution of T-cell development by Pax5-deficient B-cell progenitors. *Nature* **401**: 603–606.
- Saunders, D., Lucas, K., Ismaili, J., Wu, L., Maraskovsky, E., Dunn, A., and Shortman, K. 1996. Dendritic cell development in culture from thymic precursor cells in the absence of granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* **184**: 2185–2196.
- Schmitt, T.M., de Pooter, R.F., Gronski, M.A., Cho, S.K., Ohashi, P.S., and Zuniga-Pflucker, J.C. 2004. Induction of T cell development and establishment of T cell competence from embryonic stem cells differentiated in vitro. *Nat. Immunol.* **5**: 410–417.
- Singh, H., Medina, K.L., and Pongubala, J.M. 2005. Contingent gene regulatory networks and B cell fate specification. *Proc. Natl. Acad. Sci.* **102**: 4949–4953.
- Sitnicka, E., Bryder, D., Theilgaard-Monch, K., Buza-Vidas, N., Adolfsson, J., and Jacobsen, S.E. 2002. Key role of flt3 ligand in regulation of the common lymphoid progenitor but not in maintenance of the hematopoietic stem cell pool. *Immunity* **17**: 463–472.
- Souabni, A., Cobaleda, C., Schebesta, M., and Busslinger, M. 2002. Pax5 promotes B lymphopoiesis and blocks T cell development by repressing Notch1. *Immunity* **17**: 781–793.
- Urbanek, P., Wang, Z.Q., Fetka, I., Wagner, E.F., and Busslinger, M. 1994. Complete block of early B cell differentiation and altered patterning of the posterior midbrain in mice lacking Pax5/BSAP. *Cell* **79**: 901–912.
- Veiby, O.P., Lyman, S.D., and Jacobsen, S.E. 1996. Combined signaling through interleukin-7 receptors and flt3 but not c-kit potently and selectively promotes B-cell commitment and differentiation from uncommitted murine bone marrow progenitor cells. *Blood* **88**: 1256–1265.

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