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Editorial: **All that you can B: mirn23a regulates B versus myeloid fates**

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Since their discovery [1, 2], miRNAs (or mirns) have steadily gained appreciation as key regulators of gene expression in virtually all eukaryotic cells. These small, noncoding RNAs regulate their target genes through a variety of mechanisms, including transcript turnover and translational rates. In many cases, miRNAs control cellular differentiation programs and may be active in both early development and later, in affecting the function of differentiated cells. Not surprisingly, miRNAs play roles in lymphocyte biology, and within the B lineage, they have been implicated in development, maturation, and function. In this issue of *JLB*, the Dahl laboratory [3] reports results from a knockout mouse that show mirn23a is a key epigenetic regulator of the B versus myeloid fate decision during hematopoiesis (**Fig. 1**).

Whereas transcription factors governing B-lineage specification and commitment have been known for several decades, their complex interplay with epigenetic modifiers and miRNA networks are only now gaining appreciation [4]. For example, the transcription factors PU.1 and Ikaros are well-established players in early B lymphopoiesis. PU.1 is expressed in HSCs, as well as myeloid and lymphoid progenitors, and shifts in PU.1 expression yield altered developmental outcomes, generally favoring higher myeloid-to-lymphoid ratios. HSCs give rise to a CLP pool, which includes a BLP subset. These, in turn, yield the cells in which Ig gene rearrangement begins, termed prepro-B or early pro-B (Hardy fraction A). Ig heavy- and light-chain rearrangements continue through subsequent intermediate and late pro-B stages (Hardy fractions B and C), followed by large (cycling) and small pre-B stages (Hardy fractions C′ and D, respectively), and culminate in the immature B cell, characterized by surface expression of a complete BCR (Hardy fraction E). Transcription factors E2A, FoxO1, EBF1, and Pax5 orchestrate B cell commitment and development through these stages, and several miRNAs play regulatory roles. For example, the miR-17~92 cluster and miR-34a are key players in the pro- to pre-B cell transition [4], and the miR-212/132 cluster targets the Sox4 transcription factor, likely controlling pro-B cell survival and proliferation [5].

Another miRNA cluster, mirn23a (also known as MiRNA23a), was previously identified in a cell-line screen for miRNAs induced by PU.1 [6]. When overexpressed, mirn23a mimicked the ability of PU.1 to promote myeloid differentiation at the expense of B lymphopoiesis. Despite these tantalizing in vitro observations, the role of mirn23a in vivo

Abbreviations: BLP = B cell-biased lymphoid progenitor, CLP = common lymphoid progenitor, CMP = common myeloid progenitor, EBF1 = early B cell factor 1, FoxO1 = forkhead box O1, GMP = granulocyte/monocyte progenitor, HSC = hematopoietic stem cell, LMPP = lymphoid-primed multipotent progenitor, Mef2c = myocyte enhancer factor 2c, MEP = megakaryocyte/erythroid progenitor, miR/mirn/miRNA = microRNA, MPP = multipotent progenitor, MSCV = murine stem cell virus, Pax5 = paired box 5, Trib1/2/3 = Tribbles 1/2/3

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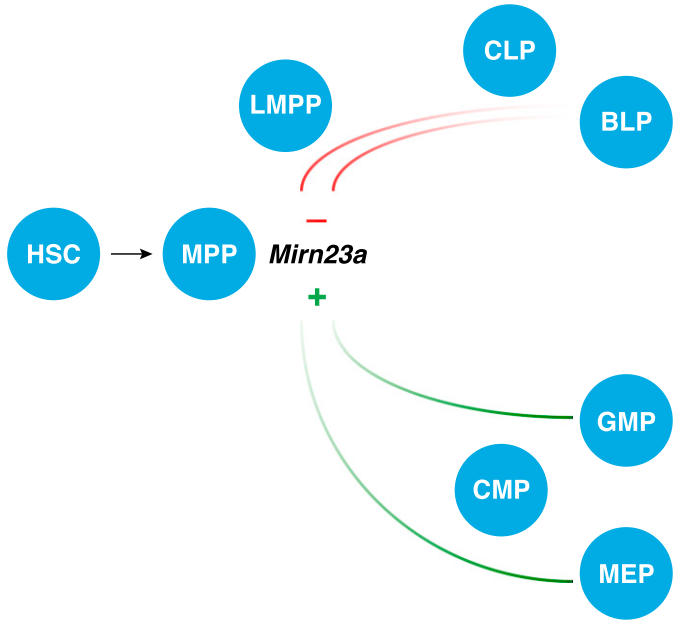

Figure 1. The work of Kurkewich et al. [3] indicates mirn23a acts within and/or immediately following the multipotent progenitor (MPP) pool, resulting in diminished generation of the B cell biased lymphoid progenitor subset (BLP) of common lymphoid progenitors (CLP), but fostering production of common myeloid progenitors (CMP) as well as granulocyte/monocyte and megakaryocyte/erythroid progenitors (GMP, MEP).

remained speculative until now. The germline knockout mouse generated by Kurkewich et al. [3] is missing the mirn23a miRNA cluster, which includes 3 species: miR-23a, miR-24-2, and miR-27a. Homozygous mirn23a knockout mice are overtly healthy and fertile, perhaps because miR-24 is expressed in these mice by a homologous mirn23b gene, albeit at very low levels compared with wild-type, suggesting it does not fully compensate for the absence of the mirn23a gene. Moreover, unlike mirn23a, mirn23b is expressed at low levels in hematopoietic cells. The findings presented by these investigators include ex vivo observations of progenitor, developing, and mature B cell subsets in mirn23a knockout mice, as well as in vitro differentiation analyses. Together, the observations demonstrate that mirn23a antagonizes B lymphopoiesis, at least in part via regulation of Trib3 pseudokinase.

Kurkewich et al. [3] first trace early B lymphopoiesis in the bone marrow of 5- to 6-wk-old wild-type and knockout mice. Overall, mirn23a knockouts have increased numbers of bone marrow B cells and reduced myeloid cells, with no change in erythroid production. Closer inspection reveals that all developing B cell subsets are significantly increased in proportion and number, including prepro-B, pre-B, pro-B, and immature B cell pools (Hardy fractions B–E). Importantly, the numbers and representation of HSCs and the earliest specified progenitors (MPP and LMPP) are unchanged in mirn23a$^{-/-}$ bone marrow, but the BLP subset of CLPs is increased significantly, consistent with increased numbers of subsequent developing B cell subsets. Conversely, the CMP, GMP, and MEP subsets are all decreased significantly in number, implying that the increase in CLPs is at the expense of myeloid progenitors in these mice. Shifts consistent with increased bone marrow B cell and reduced myeloid cell generation are observed in the periphery of mirn23a null mice, with significant increases in early transitional and follicular B cell subsets and significant reductions in the number of CD11b^{+} myeloid cells. CD4 and CD8 T cells in thymus and spleen do not differ between wild-type and mirn23a knockouts, nor do splenic marginal zone B cell numbers. Serum IgG levels did not differ significantly between mirn23a null and sufficient mice, although the knockouts show a 1.5-fold increase in bone marrow plasma cells, suggesting no substantial defect in antibody formation, although induced responses have not yet been interrogated in this system. A further indication that mirn23a-deficient B cells retain normal function is that they divide and differentiate into short-lived plasma cells after in vitro stimulation. The authors also note that homozygous knockouts have no gross defect in T cell immunity following *Leishmania* challenge.

Cell culture studies further support the notion that mirn23a counteracts B cell differentiation from a multipotent progenitor, while facilitating the myeloid lineage. Kurkewich et al. [3] coculture sorted progenitor cells with OP9, a bone marrow stromal cell line that supports differentiation of all hematopoietic lineages except T cells [7]. Lin^{-} progenitors from mirn23a$^{-/-}$ mice in OP9 cocultures show enhanced differentiation of B220^{+} cells at the expense of CD11b^{+} cells. In addition, multipotent (erythroid-myeloid-lymphoid) cells, stably transduced with MSCV-mirn23a, showed dramatically reduced differentiation of B220^{+} cells in the coculture system.

Kurkewich et al. [3] also make progress toward identifying the targets regulated by mirn23a. Transcriptional studies used RNA isolated from primary progenitor cells and from cell lines transduced with MSCV or MSCV-mirn23a. In these studies, mirn23a repressed Ikaros (*Izkf1*) and FoxO1 (known miR-27a targets), as well as Ebf1, Pax5, and Mef2c, but did not affect transcription factor 3 (E2A) expression. In addition, these studies indicate that expression of the miR-24 target, Trib3, is also repressed by mirn23a and that Trib3 overexpression in wild-type cells phenocopies the behavior of mirn23a-deficient cells. Unlike Trib1 and Trib2, which are differentially expressed in several progenitors and lineages, the Trib3 homolog has not heretofore been implicated in regulating hematopoiesis [8]. Thus, although further investigation is required, this observation suggests a previously unappreciated role for Trib3.

In toto, this report confirms mirn23a as a regulator that limits B lymphopoiesis and fosters myeloid cell production. The findings prompt a model that posits an increase in mirn23a expression within MPP cells, possibly working in concert with PU.1, which enables skewing of

subsequent pools toward CMPs and away from B cell progenitors. This work further suggests a mechanism whereby members of the mirn23a complex target "upstream" transcription factors, such as Ikaros, as well as the Trib3 pseudokinase, which in turn, lead to the down-regulation of transcription factors such as Ebf1 and Pax5.

The mirn23a null mouse will likely prove a valuable tool for understanding phenomena such as hematopoietic lineage divergence, the control of B cell maturation/differentiation in the periphery, and shifts in B lymphopoiesis and B cell function associated with age. A primary example involves the long-standing debate regarding where and when the myeloid, T, and B lymphoid lineages diverge during hematopoiesis. The conventional model (Fig. 1) posits that HSCs yield an MPP population comprised of cells with similar, if not identical, potential to give rise to myeloid or lymphoid precursors. Thus, the induction of gene-expression programs unique to each pathway would occur after or coincident with exit from the MPP pool. With this viewpoint in mind, given that mirn23a deletion results in fewer myeloid precursors and increased early B-lineage precursors, these results would appear to indicate that mirn23a regulates distinct gene-expression networks and hence, distinct developmental events in early myeloid- and lymphoid-committed progenitors. Alternatively, MPPs and other multipotent precursors may harbor cells that are biased toward a particular developmental fate well before firm commitment to the corresponding lineage. Consistent with this possibility, several past reports suggest that batteries of early B-lineage-affiliated genes are first expressed in CLPs and MPP-proximal progenitors, known as LMPPs [9, 10]. Indeed, early reports along this line indicated that the Mb1 gene, encoding the B-lineage signaling protein Ig-α, is first expressed in LMPP cells [10]. Furthermore, deletion of a B-lineage-restricted enhancer element within the RAG locus affects variable, diversity, and joining recombination in multipotent CLPs without affecting T cell development [11], and early T cell precursors can develop despite genetic mutations that prevent the generation of CLPs [12]. Thus, an alternative possibility is that mirn23a regulates early gene-expression patterns within the MPP pool that establish early developmental biases for the myeloid and lymphoid fates. Additional work dissecting the role of mirn23a in T cell development may shed light on this important issue.

The extent to which mirn23a influences mature and antigen-experienced B cell pools is not yet clear. Although the preimmune transitional and follicular B cell numbers seem to be increased in mirn23a knockouts, the use of 5- to 6-wk-old mice precludes establishing whether this reflects more rapid progress to normal steady-state numbers or whether the replete mature pools will also be abnormally enlarged in adults (>3 mo old). Likewise, whereas mirn23a apparently does not grossly alter normal B cell function in the periphery, this question remains to be addressed by in vivo antigen/pathogen challenge. There is an emerging picture for molecular control of germinal center formation and maintenance that involves the interplay of transcription factors, miRNAs, and chromatin modifications, including a role for PU.1 and other agents that act during B lymphopoiesis [13]. Accordingly, the mirn23a complex may also play key roles in activated and antigen-experienced B cell subsets. Finally, further work with the mirn23a null mouse may provide insight into the mechanistic bases for several age-associated changes. For example, myeloid bias in bone marrow output becomes greater with age in both mice and humans; furthermore, bone marrow B cell output steadily wanes with advancing age. As a first step, it will be important to determine whether mirn23a knockouts retain a higher lymphoid-to-myeloid ratio, increased BLP numbers, and increased bone marrow B cell output as they age.

Overall, the findings reported by Kurkewich et al. [3] broaden appreciation for how miRNAs govern compartments fed by the HSC pool and prepare fertile ground for future studies in this exciting area.

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