Supplemental Information

MicroRNA-34a Perturbs B Lymphocyte Development by Repressing the Forkhead Box Transcription Factor Foxp1

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miR-34a expression (arbitrary units)

Vector

miR-34a

FUGW/34a

UbC | GFP | miR-34a | WPRE

FUGW | FUGW/34a

CD19

GFP

EtBr

Rao et al, Figure S1
A
miR-34a expression
(normalized to 5S)

B
Foxp1 RNA
(fold over control)

C
Pro-B cells (% of GFP+ Cells)

D
Pre-B Cells (% of GFP+ Cells)

Rao et al, Figure S2
Rao et al, Figure S3
Rao et al, Figure S4

A

MIG/Foxp1

MIG/Foxp1+34a

B

GFP+IgM-SSC-low:

Vector

miR-34a

Foxp1

Foxp1+34a

CD19

CD43

B220

GFP

Cell number

GFP+IgM-SSC-low:

Vector

miR-34a

Foxp1

Foxp1+34a

20.48 48.8
1.64 29.08
51.05 40.03
46.30 28.73
31.49 35.60
1.37 23.60
2.11 30.80
A

MIG/BCL2

LTR BCL2 IRES GFP LTR

MIG/Bcl-2+34a

LTR BCL2 34a IRES GFP LTR

B

![Graph showing miR-34a expression](image)

C

![Graph showing BCL2 expression](image)

D

**GFP+IgM-SSC-low:**

- **BCL2**
- **BCL2+34a**

E

**GFP+IgM-SSC-low:**

- **BCL2**
- **BCL2+34a**
Supplemental Figure Legends.

Figure S1. In vitro analyses of vectors used in this study.

A. RT-qPCR analysis of expression of miR-34a from the vector described in Figure 2 shows overexpression following transduction of 70Z/3 cells.

B. Flow cytometric analysis of GFP expression in 70Z/3 cells following transduction with either MGP(vector) or MGP/34a (miR-34a).

C. Schematic representation of lentiviral construct used to express miR-34a in human cell lines. A ubiquitin promoter drives expression of GFP and miR-34a (Lois et al., 2002).

D. miR-34a expression was measured by RT-qPCR in NALM6 human pre-B cells following lentiviral transduction of FUGW or FUGW/miR-34a.

E. Flow cytometric analysis of GFP expression in NALM6 cells following lentiviral transduction of either FUGW or FUGW/miR-34a.

F. Northern Blot analysis of miR-34a expression in NALM6 cells transduced with the lentiviral vectors, FUGW and FUGW/miR-34a.

Figure S2. Low-level miR-34a expression has subtle impacts on B-cell development.

A. miR-34a expression was measured in bone marrow samples of mice receiving MGP-transduced (vector) or MGP-miR-34a-lo (miR-34a-lo) vectors. As described in the methods section, miR-34a-lo contains the endogenous stem-loop structure and 5’ and 3’ flanking regions found in the genomic sequence of miR-34a. This results in production of lower amounts of mature miR-34a (n=4 for each group). The difference depicted is statistically significant (T-test; p=0.019).
B. Foxp1 expression was measured in the bone marrow by RT-qPCR in the same mice as in A. A modest reduction in Foxp1 levels is seen, but less than that observed in mice overexpressing miR-34a at high levels. The difference depicted is statistically significant (T-test; p=0.018).

C. Enumeration of pro-B cells in mice described in A. There is an elevation of pro-B cells, but results are not statistically significant. This experiment was repeated three times, with similar trends being observed. The difference depicted is not statistically significant (T-test; p=0.15).

D. Enumeration of pre-B cells in mice described in A. There is a mild decrease in pre-B cells, but the results are not statistically significant (p=0.08).

**Figure S3. Bcl2 inhibition does not recapitulate the B-cell phenotype induced by miR-34a.**

A. Vector design for MGP/Bcl2si which produces a siRNA that targets Bcl-2.

B. Bcl-2 expression in murine bone marrow measured by RT-qPCR, in mice that received marrow transduced with MGP (vector) or MGP/miR-34a (miR-34a). This is the result from a representative experiment (n=4; T-test, p=0.03). A second experiment showed a similar trend.

C. Bcl-2 expression in murine bone marrow, measured by RT-qPCR in MGP- or MGP-Bcl2si-transduced mouse bone marrow. As expected, there is knockdown of Bcl2.

D-G. Enumeration of pro-B cells (D; T-test, n.s.), pre-B cells (E; T-test, p=0.006), mature B-cells (F; T-test, p=0.008), and myeloid cells (G; T-test, p=0.0075) in mice receiving
marrow transduced with MGP (Vector) or MGP-Bcl2si (Bcl2-si). Note that all B-cell lineages and myeloid lineage cells show either a trend towards reduction or statistically significant reduction in the marrows that express Bcl2-si.

**Figure S4. Foxp1 cDNA rescues the phenotype caused by miR-34a.**

A. Flow cytometric analysis of bone marrow from recipient mice transduced with MIG, MIG/Foxp1, or MIG/Foxp1+34a. Shown is a histogram of GFP fluorescence. Note that the mean fluorescence as well as the size of the positive population is lowered by the addition of Foxp1 to MIG and is further lowered by the addition of the miR-34a element.

B. Flow cytometry histograms of GFP+ cells analyzed for pro-B cell markers (B) and pre-B cell markers (C). The increased number of pro-B cells seen in miR-34a mice (CD19+ c-kit+) is not seen in Foxp1-expressing or Foxp1+34a expressing mice (B). The decreased number of pre-B cells (CD43-B220+) observed in miR-34a mice is not seen in Foxp1-expressing or Foxp1+34a expressing mice (C).

**Figure S5. Bcl2 does not rescue the B-cell developmental block induced by miR-34a.**

A. Schematic representation of constructs encoding BCL2 and BCL2+miR-34a.

B. miR-34a expression in MIG (vector), MGP/34a (miR-34a), MIG/BCL2(BCL2) and MIG/BCL2+34a (BCL2+34a)-transduced mice, as measured by RT-qPCR.

C. BCL2 expression, measured by RT-qPCR in the same mice as in (B).

D. Bone marrow cells stained with CD19, c-kit and IgM, analyzed by flow cytometry. Left hand panels show representative histograms of the GFP+ IgM-negative compartment in BCL2 and BCL2+34a expressing mice. The right hand panel shows data enumerating pro-B cells (CD19+IgM-c-kit+) as a percentage of total GFP+ cells.
(n=4). The number of pro-B cells was not significantly different in BCL2 mice versus control, but was significantly increased in BCL2+34a mice (T-test, p=0.0003).

E. Bone marrow cells stained with B220, IgM and CD43 were analyzed by flow cytometry. Left hand panels show representative histograms of the GFP+ IgM- compartment in BCL2 and BCL2+34a expressing mice. The right hand panel shows data enumerating pre-B cells (B220+CD43-IgM-) as a percentage of the total GFP+ cells (n=4). The number of pre-B cells was not significantly different in BCL2 mice versus control, but was significantly increased in BCL2+34a mice (T-test, p=0.004).
Table S1. Cloning primers and template oligos. Lowercase lettering is used to show the spacer sequences in the oligonucleotide sequences for building MGP/anti-34as. Also shown in lowercase is the entire cDNA sequence for Foxp1 that was cloned into the MIG vector as described in the methods section.

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**Foxp1 cDNA sequence**

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