Transgenic Expression of MicroRNA-185 Causes a Developmental Arrest of T Cells by Targeting Multiple Genes Including Mzb1

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MicroRNAs (miRs) are small noncoding RNAs (20–24 nucleotides in length) that regulate gene expression by causing mRNA degradation and/or translational inhibition (1, 2). Functional roles of the miRs in the immune system are being partly elucidated with loss- and gain-of-function approaches. For example, conditional knock-out lines of Dicer, an RNase III enzyme critical for miR biogenesis, in thymic epithelial cells causes a premature thymic involution, in part via the diminished expression of miR-29 (3). The elimination of Dicer at the DN3 stage (CD4−CD8−CD44−CD25+) of thymocytes reduces thymic cellularity (4). For pro-B cells, this knock-out impairs their transition to pre-B cells, whereas its loss in mature CD19+ B cells causes B cell autoantibody production (5, 6). This was linked to the loss of miR-185 and the up-regulation of its target, Bruton’s tyrosine kinase (Btk) (5). Gain-of-function approaches have uncovered roles for miR-146 and miR-155 in regulating IL-2 production in T cell and increasing NK cell numbers and cytokine production, respectively (7, 8). miR-185 is a stress-responsive miR expressed in the thymus (9). It is encoded on human chromosome 22q11.2 and is haploinsufficient in 22q11.2/DiGeorge syndrome patients (10). Such patients can have a thymic hypoplasia, hypoparathyroidism, cardiac anomalies, and/or learning disabilities (11). Some patients have an increased frequency of autoimmune disorders and T helper cell alterations (12, 13). One-third of 22q11.2 deletion syndrome patients will develop schizophrenia as adults (14). Individuals with a duplication of chromosome 22q11.2 (trisomy 22q11.2) can have similar clinical presentations, suggesting that both reductions and elevations in miR-185 are clinically informative.

miR-185 is a microRNA (miR) that targets Bruton’s tyrosine kinase in B cells, with reductions in miR-185 linked to B cell autoantibody production. In hippocampal neurons, miR-185 targets both sarcoplasmic/endoplasmic reticulum calcium ATPase 2 and a novel Golgi inhibitor. This miR is haploinsufficient in 90–95% of individuals with chromosome 22q11.2 deletion syndrome, patients who can present with immune, cardiac, and parathyroid problems, learning disorders, and a high incidence of schizophrenia in adults. The reduced levels of miR-185 and its target, Bruton’s tyrosine kinase, which results in a peripheral T cell lymphopenia and an immune, cardiac, and parathyroid problems, learning disorders, and a high incidence of schizophrenia in adults. The reduced levels of miR-185 and its target, Bruton’s tyrosine kinase, which results in a peripheral T cell lymphopenia and unusual T helper cell skewing. The molecular targets of miR-185 are unknown. Using an miR-185 T cell transgenic approach, increasing levels of miR-185 attenuated T cell development via the targeting of Mzb1, Nfatc3, and Camk4. This caused a peripheral T cell lymphopenia. Mzb1, Nfatc3, and Camk4 are identified as novel targets of miR-185. Elevations in miR-185 enhanced TCR-dependent intracellular calcium levels, whereas a knockdown of miR-185 diminished these calcium responses. These effects concur with reductions in Mzb1, an endoplasmic reticulum calcium regulator. Consistent with their haploinsufficiency of miR-185, Mzb1 levels were elevated in thymocyte extracts from several 22q11.2 deletion syndrome patients. Our findings indicate that miR-185 regulates T cell development through its targeting of several mRNAs including Mzb1.

Background: MicroRNAs have critical roles in T cell development under normal and stress conditions.

Results: Increasing miR-185 levels attenuate T cell development via the targeting of Mzb1, Nfatc3, and Camk4.

Conclusion: Elevations in miR-185 impair thymopoiesis at several developmental stages.

Significance: miR-185 levels are reduced in 22q11.2 deletion syndrome patients and the identification of its gene targets is clinically informative.

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2 The abbreviations used are: miR, microRNA; CDS, coding sequence; DN, CD4−CD8−; DP, CD4+CD8−; CD4SP, CD4+CD80; CD8SP, CD4+CD8−; Btk, Bruton’s tyrosine kinase; SERCA2, sarcoplasmic/endoplasmic reticulum calcium ATPase 2; Mzb1, marginal zone B and B1-cell specific protein; Nfatc3, nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 3; CAMK4, calcium/calmodulin-dependent protein kinase IV; Mcm10, minichromosome maintenance deficient 10; Hmga1, high mobility group AT-hook 1; Igf1r, insulin-like growth factor I receptor; Dusp4, dual specificity phosphatase 4; NK, natural killer; Tg, transgenic; ANOVA, analysis of variance; TCR, T cell receptor; OVA, ovalbumin; OTII, TCR transgenic mice specific for ovalbumin peptide with I-A\*.
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micr0RNA Target Validation—miR-185 (~600-bp genomic DNA) was cloned into pCDNA3.1 (Invitrogen). The 3′- untranslated regions (3′-UTRs) of target genes (Btk, Mzb1, Mcm10, Camk4, Hmga1, Nfatc3, Igf1r, and Dusp4) were amplified by PCR and ligated into the firefly luciferase reporter construct (pMIR-REPORT, Invitrogen). Reporter constructs and a β-galactosidase vector were co-transfected into COS-1 cells (1 × 10⁵ cells/ml; 24-well plate) along with either pCDNA3.1 or pCDNA3.1-miR-185, using the FuGENE 6 transfection reagent (Roche Applied Science). Cells were processed using the luciferase assay kit (Promega). Relative luciferase activity was calculated by normalizing the firefly luciferase to the β-galactosidase. The murine Mzb1 coding sequences (CDS) were cloned into the pEF1/myc-His B plasmid (Invitrogen). Transfections were done in HEK293T cells (1 × 10⁵ cells/ml; 24-well plate) using the X-tremeGENE 9 DNA kit (Roche Applied Science). Mutations in the 3′-UTR and CDS of Mzb1 were introduced with QuikChange site-directed mutagenesis kit (Stratagene).

MicroRNA Knockdown and Immunoblotting—Jurkat T cells (2–2.5 × 10⁵ cells/ml) were transfected with either miR control inhibitor (microRNA Hairpin Inhibitor Negative Control 1, cel-miR-67) or miR-185 inhibitor (10–40 nM, miRIDIAN, ThermoFisher Scientific).
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Scientific) using PepMute siRNA transfection reagent (Signa-
gen). Immunoblotting was performed as described previously
with the following antibodies: Mzb1 (11454-1-AP, Protein-
tech), NFATc3 (SC-8321, Santa Cruz Biotechnology),
/H9252 -actin (4967, Cell Signaling), GFP (632380, Clontech), Myc epitope
(2272, Cell Signaling), anti-rabbit HRP-conjugated secondary
body, and anti-mouse IgG HRP-conjugated secondary anti-
vendor’s instructions. For the Western blots, multiple
exposures were obtained.

Measurement of Intracellular Calcium Responses—Thymo-
cytes (1 × 10^7 cells/ml) were loaded with Fluo-3-AM (4 µM,
Invitrogen) in 1X Hanks’ balanced salt solution (Cellgro) and
incubated at 37 °C for 30 min. Base-line fluorescence was mon-
tored for 45 s at 37 °C before adding biotinylated anti-CD3ε
and anti-CD4 (1 µg/ml, BioLegend). The fluorescence intensity
was measured for 45 s followed by streptavidin (2 µg/ml) and
monitoring for an additional 330 s. Jurkat T cells were stimu-
lated with the anti-clonotypic antibody, C305.2. Maximal cal-
cium responses were determined by adding ionomycin (1 µM),
which was quenched by the addition of MnCl₂ (1 mM). In cer-
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RESULTS

Elevations in miR-185 Attenuate T Cell Development—miR-185 is highly conserved and expressed in most tissues including the thymus, brain, heart, kidney, liver, lung, skin, and spleen (Fig. 1A). It is expressed in immature thymocytes, mature CD4⁺CD8⁻ and CD4⁻CD8⁺ T cells, and B cells (5, 9, 21, 22). To determine whether miR-185 levels impact T cell functions, we utilized a gain-of-function transgenic approach (23) in which the murine primary miR-185 (pri-miR-185) was overexpressed 130-, 175-, and 250-fold in Tg-25, Tg-35, and Tg-6 lines, respectively (Fig. 1, 175-, and 250-fold in Tg-25, Tg-35, and Tg-6 lines, respectively, when compared with nontransgenic littermates (Fig. 1, A and B). Overall thymic cellularity was slightly reduced in Tg-25 and Tg-35 lines, whereas a severe thymic hypoplasia was noted in the Tg-6 line (Fig. 1E). Increasing levels of miR-185 caused a statistically significant decrease in the percentage and number of CD4⁺CD8⁻ and CD4⁻CD8⁺ SP subsets, reflected as a dramatic loss in overall thymic cellularity (Fig. 1, A–D). Elevated DN percentages in the Tg-6 line further indicated an attenuated early thymopoiesis (Fig. 1F). The Tg-6 line had no defined cortical region, an absent corticomedullary junction, and a pronounced stromal component in the medulla (data not shown).

Thymopoiesis in miR-185 Transgenic Lines Is Affected at Two Development Checkpoints—The attenuated T cell development in the miR-185 transgenic mice appeared to be during pre-TCR and TCR selection stages. To assess whether there was a defect at the pre-TCR checkpoint, CD4⁺CD8⁻ (DN) thymocytes were profiled for CD44 and CD25 cell surface expression, which marks four DN subsets, including those thymocytes at pre-TCR selection stage (DN3). Increasing miR-185 levels matched the severity of the block at the DN3 (CD4⁺CD25⁻) stage, with the percentage of DN3 cells increasing from 25% in controls to 40, 51, and 73% in the miR-185 Tg-25, Tg-35, and Tg-6 lines, respectively (Fig. 2A). This was statistically significant for all the Tg lines when compared with the wild type controls, and between Tg-6 versus Tg-35 and Tg-25 (Fig. 2B). No change in intracellular TCRβ expression was detected in the DN3 subsets (Fig. 2C). To induce TCR signals, we next injected the mice intraperitoneally with anti-CD3ε. This normally causes a differentiation of DN3 thymocytes to the DP stage in Rag1-deficient mice, which is coupled with an increased thymic cellularity. The DN3 thymocytes in the miR-185 Tg-6 mice were unable to progress to the DP stage (Fig. 2, D and E). Surface expression levels of CD5 on DN3 thymocytes were normal...
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in Tg-25 and Tg-35 lines, but slightly elevated in the Tg-6 line, an indication of normal pre-TCR engagement and signal strength (data not shown). With regard to other cell populations, the percentage and number of NK T cells were similar in the miR-185 Tg lines, except for the Tg-6 line that had very low NK T cell numbers (Fig. 2F). γδ T cell numbers in all Tg lines were similar to the control, whereas their percentages were slightly increased (Fig. 2G).

The reduced SP thymocytes in all the miR-185 Tg lines suggested an impairment of positive selection. Consistent with this, the percentages of CD69⁺ TCRβhigh total thymocytes, including CD69⁺ TCRβhigh CD4 and CD8 SP thymocytes, were reduced in a statistically significant manner (Fig. 3, A and B). The reduction in positive selection was consistent with an increased percentage of DP thymocytes that were CD69⁺ TCRβhigh (Fig. 3, C and D). Differences between the
Elevated levels of miR-185 cause a peripheral T cell lymphopenia. A, flow cytometric analysis of CD4+ and CD8+ T cells in the lymph nodes from normal and miR-185 Tg mice. B, bar graphs show absolute numbers of CD4+ and CD8+ T cells in the lymph nodes in the control and miR-185 Tg mice. Data are of the mean ± S.E. from WT (n = 40), Tg-25 (n = 16), Tg-35 (n = 46), and Tg-6 (n = 59) mice (*, p < 0.05, **, p < 0.01, ***, p < 0.001; one-way ANOVA followed by Tukey’s post hoc test). C, relative mean fluorescence intensity (MFI) levels ± S.E. of CD25, CD44, and CD62L markers on CD4+ T cells in the lymph nodes of WT (white), Tg-25 (light gray), Tg-35 (dark gray), and Tg-6 (black) mice using at least five mice per group (n.s. = nonsignificant, *, p < 0.05, **, p < 0.01, ***, p < 0.001; one-way ANOVA followed by Tukey’s post hoc test). D, graph represents relative IL-2 secretion from anti-CD3e/CD28 stimulated total CD4+ T cells in miR-185 Tg lines and the control (WT), set to 1. Each bar is the mean ± S.E. of at least five independent experiments (n.s. = nonsignificant, *, p < 0.05, **, p < 0.01, ***, p < 0.001; two-tailed unpaired Student’s t test). E, lymphocytes from the lymph nodes of OTII Tg and OTII/miR-185 Tg mice were stained for CD4 and CD8 and analyzed by FACS. F, average percentages of CD4+ and CD8+ T cells in the lymph nodes. G, surface expression of TCR Vα2 gated on CD4+ and CD8+ T cells from the lymph nodes of OTII Tg (dark gray) and OTII/miR-185 Tg mice (black line). H, relative mean fluorescence intensity (MFI) levels of TCR Vα2 on CD4+ and CD8+ lymphocytes. E–H, data are of at least six mice per group. Bar graphs represent the mean ± S.E. values (*, p < 0.05, **, p < 0.01, ***, p < 0.001; two-tailed unpaired Student’s t test). I, graph shows absolute numbers of natural regulatory T (nTreg) cells in the spleen of the control and miR-185 Tg mice. Data are of the mean ± S.E. from at least three mice per group (n.s. = nonsignificant, *, p < 0.05, **, p < 0.01, ***, p < 0.001; one-way ANOVA followed by Tukey’s post hoc test).

Closely matched Tg-25 and Tg-35 lines were also of statistical significance when the reductions in CD4+CD8− and CD4−CD8+ subsets were compared (Fig. 3, C and D). Negligible numbers of mature CD69−TCRβhigh SP thymocytes were found in the miR-185 Tg-6 line (Fig. 3, C and D). Moreover, CD5 expression on CD69−TCRβhigh DP thymocytes was lower in miR-185 Tg lines, supporting impaired positive selection (Fig. 3, E and F). Attenuated positive selection was further established by comparing OTII-specific TCR transgenic T cells developing in the miR-185 Tg-35 lines. Their numbers were significantly reduced in the OTII/miR-185 Tg-35 double Tg lines when compared with the OTII Tg parental line (Fig. 3, G and H), and those residual SP cells lost the expression of the transgenic TCRα subunit (Fig. 3, I and J). Severe loss of CD4 SP thymocytes in the OTII/miR-185 Tg-35 line could reflect enhanced negative selection. However, the total number of thymocytes was equivalent in both the OTII and the OTII/miR-185 Tg-35 lines (Fig. 3K). Furthermore, in vitro treatment of thymocytes from the OTII/miR-185 Tg-35 line with an OVA class II peptide induced DP cell death, indicating that negative selection was intact and similar (Fig. 3L). The impairment at the DN3 and DP stages was not due to increased cell death because annexin V+ percentages were similar in the miR-185 Tg lines (Fig. 3M). Taken together, these findings demonstrate that increases in miR-185 reduced the effectiveness of TCRβ and positive selection.

The miR-185 Transgenic Lines Have a Peripheral T Cell Lymphopenia—An miR-185 dose-dependent reduction in the percentage and number of mature peripheral CD4+CD8− and CD4−CD8+ T cells occurred in all three miR-185 Tg lines and was most pronounced in Tg-6 (Fig. 4, A and B). Peripheral T cells from all the miR-185 Tg lines displayed spontaneous
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### TABLE 1

The top 25 down-regulated genes with predicted miR-185 binding sites on their 3’-UTR and/or coding sequences.

This table provides fold changes of the down-regulated genes in miR-185 Tg DN3 thymocytes as compared with the wild type control. miRWalk target prediction database was used to identify putative miR-185 target genes.

| Gene Symbol | Fold | Description |
|-------------|------|-------------|
| Mzb1        | -4.84| Marginal zone B and B1-cell specific protein |
| HK2         | -4.19| Hexokinase II |
| Hoxa7       | -3.81| Homeobox A7 |
| Tpt1        | -3.79| Protein-tyrosine sulfotransferase 1 |
| Mcm10       | -3.26| Minichromosome maintenance deficient 10 |
| Camk4       | -3.24| Calcium/calmodulin-dependent protein kinase IV |
| Stab1       | -3.20| Stabilin 1 |
| E130012A19Rik | -3.10| RIKEN cDNA E130012A19 gene |
| 231004H10Rik | -3.06| RIKEN cDNA 231004H10 gene, MIRTA22 |
| Abcf2       | -2.94| ATP-binding cassette, subfamily F (GCN20), member 2 |
| Gja1        | -2.75| Gap junction membrane channel protein alpha 1 |
| Gga2        | -2.73| Golgi-associated, gamma adaptin ear-containing, ARF-binding protein 2 |
| Nsf         | -2.59| N-Ethylmaleimide sensitive fusion protein |
| Cdc53       | -2.52| Coiled-coil domain containing 53 |
| Msn1        | -2.41| Meiosis-specific nuclear structural protein 1 |
| Ncl         | -2.40| Nucleolin |
| Huma1       | -2.38| High mobility group AT-hook 1 |
| Shmt1       | -2.36| Serine hydroxymethyltransferase 1 |
| Mcm5        | -2.26| Minichromosome maintenance deficient 5 |
| Igfbp3      | -2.22| Insulin-like growth factor 2 mRNA binding protein 3 |
| Nfatc3      | -2.21| Nuclear factor of activated T cells, cytoplasmic, calcineurin dependent 3 |
| Rrm1        | -2.19| Ribonucleotide reductase M1 |
| Sce12       | -2.13| Suppressor of zeste 12 homolog (Drosophila) |
| Igf1r       | -2.14| Insulin-like growth factor receptor |
| Rd1         | -2.13| RNA terminal phosphate cyclase-like 1 |

Quantitative RT-PCR with gene-specific probes for Mzb1 mRNA, one actually in the coding sequence (Fig. 5D). In transfection assays with just the CDS of Mzb1, miR-185 reduced its expression when compared with the vector control in a statistically significant manner (Fig. 5, E and F). Mutating the target sequence in Mzb1 prevented its down-regulation (Fig. 5, E and F). To confirm targeting of the 3’-UTR of Mzb1, luciferase reporter assays were performed. Relative to the control, the luciferase activity of Mzb1 3’-UTR was decreased more than 2-fold (Fig. 5G). Mutations of the target sequence within the Mzb1 3’-UTR restored luciferase levels (Fig. 5G). A number of additional targets identified in the microarray (Mcm10, Camk4, Huma1, Nfatc3, Igf1r, and Dusp4) were validated as novel miR-185 targets in luciferase reporter assays, with the Btk 3’-UTR included as a positive control (Fig. 5H).

miR-185 Levels Affect TCR-driven Intracellular Calcium Responses—One of the miR-185 targets, Mzb1, is an endoplasmic reticulum-associated protein that regulates B cell receptor-driven calcium responses (25). We examined the changes TCR-driven intracellular Ca$^{2+}$ responses in DP thymocytes from the miR-185 Tg lines. DP thymocytes from normal mice increased intracellular Ca$^{2+}$ responses 30–40 s after TCR/CD4 cross-linking (Fig. 6A). The magnitude of the TCR-induced Ca$^{2+}$ response was significantly higher in the Tg-25 and Tg-35 lines (Fig. 6, A and B). The addition of the calcium ionophore, ionomycin, revealed an identical capacity of all groups of thymocytes to internalize Ca$^{2+}$, indicating that the differences in the cells were TCR signaling-dependent (Fig. 6C). Thymocytes with varying levels of miR-185 exhibited similar Ca$^{2+}$ responses following thapsigargin addition (Fig. 6D). These experiments indicated that all the Tg lines had similar endoplasmic reticulum Ca$^{2+}$ stores as wild type mice. In a loss-of-function approach, chemically modified miR inhibitors (antagomirs) were used to reduce miR-185 activity in Jurkat T cells. Inhibiting miR-185 increased Mzb1 protein expression in a dose-dependent and statistically significant manner when compared with the control inhibitor (cel-miR-67) (Fig. 6, E and F). The knockdown of miR-185, which resulted in higher Mzb1 levels, reduced TCR-mediated intracellular calcium levels in Jurkat T cells (Fig. 6, G and H). In summary, miR-185 directly affects TCR-triggered calcium responses in developing thymocytes and Jurkat T cells.
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**DISCUSSION**

Gain- and loss-of-function approaches were used to characterize the function role of miR-185 in T cells and identify its mRNA targets. A transgene-driven overexpression of miR-185 caused a developmental impairment in thymopoiesis.

The most down-regulated target, Mzb1, was first identified as a novel gene induced during B to plasma cell differentiation, regulating proper assembly and secretion of mature IgM (26, 27). It is highly expressed in marginal zone B cells and regulates intracellular Ca\(^{2+}\) flux upon B cell receptor stimulation (25). Our findings indicate that Mzb1 is also highly expressed in DN3 thymocytes and is present in Jurkat T cells, consistent with prior Northern blotting results (26–28). miR-185 targets two highly conserved sites in Mzb1, one in the CDS. This likely
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FIGURE 6. miR-185 controls TCR-stimulated intracellular calcium responses. A, intracellular calcium flux was analyzed by flow cytometry in DP thymocytes from the WT (black line), Tg-25 (blue line), and Tg-35 (red line) mice. DP thymocytes were gated by size. Black arrows indicate the time points for each treatment. Flu-3 AM-loaded thymocytes were treated with biotinylated (Bio) anti-CD3ε and anti-CD4 followed by streptavidin (SA), ionomycin (Iono), and MnCl₂. B, graph shows relative changes in TCR-triggered peak Ca²⁺ influx over the base line. Each bar represents the mean ± S.E. of six independent experiments (*, p < 0.05, **, p < 0.01, ***, p < 0.001; two-tailed unpaired Student's t test). C and D, Flu-3 AM-loaded thymocytes were treated with ionomycin (Iono) (C) and thapsigargin (Thapsi) (D) in the absence of extracellular calcium. DP thymocytes were gated electronically. Experiments were repeated two times, and representative plots are shown from the WT (black line), Tg-25 (blue line), and Tg-35 (red line) mice. E, representative immunoblot shows Mzb1 expression in Jurkat T cells transfected with varying concentrations of miR-185 inhibitor (a-miR-185) when compared with the control (miR negative control inhibitor). β-Actin was used as the endogenous control. Band intensities of Mzb1 and β-actin were measured using the ImageJ software. The relative amounts of Mzb1 protein were shown as normalized to the control inhibitor, which was set as 1. This was done by dividing the Mzb1/β-actin ratio of each sample to that of the control sample. F, graph represents the mean ± S.E. of relative Mzb1 levels in Jurkat T cells transfected with the control and miR-185 inhibitor from five independent experiments, performed in at least duplicates (*, p < 0.05, **, p < 0.01, ***, p < 0.001; two-tailed unpaired Student's t test). G, intracellular calcium responses were analyzed by flow cytometry over time in Jurkat T cells following transfection with a-miR-185 (red line) and the control inhibitor (black line). Flu-3 AM-loaded Jurkat T cells were treated with the mAb C305.2 (C305) (anti-TCRβ), ionomycin (Iono), and MnCl₂. Black arrows indicate the time points for each treatment. H, graph shows relative changes in TCR-triggered peak Ca²⁺ influx over the base line. Each bar represents the mean ± S.E. of four independent experiments (n.s. = nonsignificant, *, p < 0.05, **, p < 0.01, ***, p < 0.001; two-tailed unpaired Student's t test).

courses to the reduced efficiency of thymopoiesis as pre-TCR- and αβ TCR-driven intracellular calcium responses are likely too high to support expansion or positive selection, respectively. Reductions in NFATc3 and CAMK4 also affect these pathways. In fact, the targeted elimination of Mcm10, Hmga1, and Dusp4 were additional targets. miR-185 can target Six1, Igf1r, and Cdc42, genes involved in controlling cell cycle progression in various cancer cells (32–34). Interestingly, although these were not identified in our thymocyte screen, they may play a role in the peripheral T cell lymphopenia (29, 30).

Our data raise important questions as to whether miR-185 affects T and B cell functions in humans. Patients with 22q11.2 deletion syndrome, haploinsufficient for miR-185, have an increased prevalence of autoimmune disorders and B cell defects (10). We suggest that reductions in miR-185 affect the expression of both Btk and Mzb1 in B cells, enhancing autoantibody production (5, 25). These patients have abnormal T helper cell skewing (31). Further experiments are needed to elucidate the contribution of miR-185 in both thymocytes and thymic epithelial/mesenchymal cells. Mouse models of 22q11.2 deletion syndrome confirm a reduction of miR-185, with an age-dependent reduction in other miRs (17). A distinct miR-185 target in neurons is the calcium regulator, SERCA2 (17). Because SERCA2 is expressed at very low levels in thymocytes, the principal targets of miR-185 in thymocytes are likely distinct. In fact, Mcm10, Hmga1, Igf1r, and Dusp4 were additional targets. miR-185 can target Six1, Igf1r, and Cdc42, genes involved in controlling cell cycle progression in various cancer cells (32–34). Interestingly, although these were not identified in our thymocyte screen, they may play a role in the peripheral...
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T cells (35). It will be important to assess the consequences of both the haploinsufficiency and the trisomy of miR-185 on the novel targets reported herein as Nfatc3 and Camk4 are expressed in many tissues and organs affected by the deletions on chromosome 22q11.2.

In summary, our findings implicate a role of miR-185 in the T cell development through its targeting of genes with validated importance during thymopoiesis.

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