B-cell malignancies in microRNA Eμ-miR-17∼92 transgenic mice

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Contributed by Carlo M. Croce, September 22, 2013 (sent for review July 12, 2013)

miR-17∼92 **is a polycistronic microRNA (miR) cluster (consisting of *miR-17, miR-18a, miR-19a, miR-19b, miR-20a*, and *miR-92a*) which frequently is overexpressed in several solid and lymphoid malignancies. Loss- and gain-of-function studies have revealed the role of *miR-17∼92* in heart, lung, and B-cell development and in Myc-induced B-cell lymphomas, respectively. Recent studies indicate that overexpression of this locus leads to lymphoproliferation, but no experimental proof that dysregulation of this cluster causes B-cell lymphomas or leukemias is available. To determine whether *miR-17∼92*- overexpression induces lymphomagenesis/leukemogenesis, we generated a B-cell–specific transgenic mouse model with targeted overexpression of this cluster in B cells. The *miR-17∼92* overexpression was driven by the Eμ-enhancer and Ig heavy-chain promoter, and a 3′ GFP tag was added to the transgene to track the miR expression. Expression analysis using Northern Blot and quantitative RT-PCR confirmed 2.5- to 25-fold overexpression of all six miRs in the transgenic mice spleens as compared with spleens from wild-type mice. *Eμ-miR-17∼92* mice developed B-cell malignancy by the age of 12–18 mo with a penetrance of ∼80% (49% splenic B-cell lymphoproliferative disease, 28% lymphoma). At this stage mice exhibited severe splenomegaly with abnormal B-cell–derived white pulp expansion and enlarged lymph nodes. Interestingly, we found three classes of B-cell lymphomas/leukemias at varying grades of differentiation. These included expansion of $CD19^+$ and $CD5^+$ double-positive B cells similar to the aggressive form of human B-cell chronic lymphocytic leukemia, $B220^+$ $CD43^+$ B1-cell proliferation, and a $CD19^+$ aggressive diffuse large B-cell lymphoma–like disease, as assessed by flow cytometry and histopathological analysis.**

miR-17∼92 cluster | animal model | SLC/ABC transporters

MicroRNAs (miRs) are 21- to 22-nucleotide-long noncoding RNA molecules that regulate the expression of multiple cellular genes, and their dysregulation is involved in many human diseases including cancer. The *MiR-17∼92* cluster frequently is up-regulated in several different malignancies including diffuse large B-cell lymphoma (DLBCL) (1) and lung cancer (2, 3). The *MiR-17∼92* cluster is encoded by the chromosome 13q31 locus in humans and the 14qE4 locus in mice. This genomic region is amplified in DLBCLs and several other tumors (reviewed in ref. 4). The cluster consists of six miRs (*miR-17*, *18a*, *19a*, *20a*, *19b-1*, and *92a-1*) and has two paralogs in the genome, *miR-106a∼363* and *miR-106b∼25*, proposed to have arisen through series of duplication and deletion events during vertebrate evolution (5).

The first suggestion for its possible role in oncogenesis came from the study in Eμ-Myc mice in which enforced expression of *miR-17∼92* was shown to accelerate B-cell tumor development (6). An additional study that investigated the role of *miR-17∼92* in mice by driving its overexpression under the human *CD2* promoter in both B and T cells found that mice overexpressing the *miR-17∼92* cluster develop autoimmunity, a lymphoproliferative disease, and die prematurely (7). No lymphomas were observed in these animals, however. Targeted deletion of this cluster, but not of its paralogs, has shown that *miR-17∼92* plays an important role in B-cell development, and the KO mice die shortly after birth from lung hypoplasia and ventricular septal defects (8). Further examination of the role of individual miRs in B-cell lymphomas showed that *miR-19a* and *miR19b* are required and sufficient for the proliferative activities of the cluster (9).

To understand better the role of the *miR-17∼92* cluster in B-cell neoplastic progression, we generated *miR-17∼92* B-cell–specific transgenic mice. These mice overexpress *miR-17∼92* under the control of Ig heavy-chain promoter and Eμ enhancer and express a 3′ GFP tag to track the miRs' expression. After screening multiple founder mice, we obtained three transgenic lines that concurrently overexpress the six miRs by about 2.5- to 25-fold in their spleens as assessed by quantitative real-time PCR of purified B cells. These mice also express GFP in their B cells which serves as a proxy for *miR-17∼92* expression.

These mice develop a B-cell malignancy by age of 12–18 mo with a penetrance of ∼80% (49% splenic B-cell lymphoproliferative disease, 28% lymphoma). At this stage these mice exhibit severe splenomegaly with abnormal B-cell–derived white pulp expansion and enlarged lymph nodes. Interestingly, we found three classes of B-cell lymphomas/leukemias at varying grades of differentiation. These included expansion of $CD19^+$ and $CD5^+$ double-positive B cells similar to the aggressive forms of human B-cell chronic lymphocytic leukemia (B-CLL), $B220^+$ $CD43^+$ B1-cell

Significance

MicroRNAs (miRs) are 21- to 22-nucleotide-long noncoding RNA molecules, which regulate the expression of several genes. miR dysregulation is associated with several cancers. The MiR-17∼92 cluster is frequently upregulated in malignancies such as diffuse large B-cell lymphoma and lung cancer. To study the role of miR-17∼92 in B-cell malignancies, we developed a transgenic mouse model overexpressing this cluster in B cells. The transgenic mice developed B-cell lymphomas, and the transcriptome analysis suggested the involvement of PI3K signaling in B lymphocytes, B-cell receptor signaling, GADD45 signaling, and IL-4 signaling pathways in these disorders. Our results provide direct experimental evidence confirming that the miR-17∼92 cluster, which is amplified in human B-cell lymphoma, also is oncogenic when overexpressed in mice.

Author contributions: S.K.S., Y.P., and C.M.C. designed research; S.K.S., S.V., F.L., V.B., and Y.P. performed research; S.K.S., M.F., S.V., and Y.P. analyzed data; and S.K.S., M.F., Y.P., and C.M.C. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE50559; National Center for Biotechnology Information tracking system #16857198).

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1315365110/-/DCSupplemental.

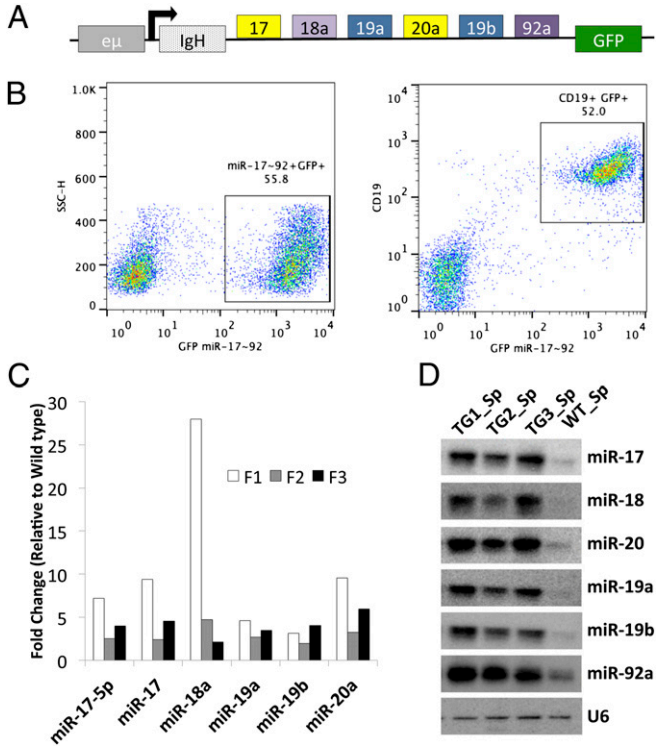

Fig. 1. (*A*) Design of the recombinant DNA construct used to generate the transgenic mice. The six miRs of the mouse *miR-17~92* cluster, *miR-17*, *miR-18a*, *miR-19a*, *miR-20a*, *miR-19b*, and *miR-92a*, were cloned under the Ig heavy-chain promoter and eμ enhancer to drive expression in B cells. The colors indicate the seed regions. The 3′ end of the construct was tagged with GFP to track the miR-expressing cells in mice. (*B*). Flow cytometry analysis of transgenic mice spleens showing GFP positivity as a marker of *miR-17~92* expression (*Left*) and its specificity to CD19$^+$ B cells (*Right*). (C) qRT-PCR analysis of spleens from transgenic mice showing *miR-17~92* overexpression in three founder lines: F1, F2, and F3. (*D*) Northern blot analysis showing robust overexpression of all six miRs in spleens from transgenic mice compared with spleens from wild-type mice. Noncoding small nuclear RNA U6 was used as a loading control.

proliferation, and a CD19$^+$ aggressive DLBCL-like disease, as assessed by flow cytometry and histopathological analysis.

Results

Generation of Eμ-miR-17~92 Transgenic Mice. To study the role of *miR-17~92* in B-cell lymphomas/leukemias, we generated mice overexpressing this miR cluster in their B cells under the Ig heavy-chain promoter and Eμ enhancer (Fig. 1*A*). The overexpression was confirmed to be B-cell specific by flow cytometry staining which showed that only CD19$^+$ B cells were positive for GFP, which was used as a proxy for the transgene detection (Fig. 1*B*). The expression of all six miRs in the cluster was assayed in transgenic mice spleens using both quantitative reverse transcription PCR (qRT-PCR) and Northern blotting, which confirmed significant up-regulation compared with wild-type mice (Fig. 1 *C* and *D*). We followed 74 transgenic mice and 30 wild-type mice as control for a 2-y period; the average age when they developed full-blown disease ranged from 9 to 12 mo. Some of these mice developed very aggressive disease accompanied by multiple tumors and enlarged spleen and lymph nodes (Fig. S1).

Eμ-miR-17~92 Transgenic Mice Develop B-Cell Lymphomas. A previous model of *miR-17~92* overexpression in mouse B and T cells reported on lymphoproliferation and autoimmunity (7). Interestingly, in our model, in which *miR-17~92* is overexpressed specifically in B cells, we observed B-cell malignancies of three kinds. Of the 74 mice, 26 developed B220$^+$CD43$^+$IgM$^+$ B1 lymphoma (Fig. 2*A*), whereas the frequency of these malignancies in transgenic mice spleens ranged from 6.9–24.7%. A total of 23 mice developed a B220$^+$CD19$^+$CD5$^+$IgM$^+$ lymphoid malignancy similar to the human B-CLL (Fig. 2*B*). Ten mice developed lymphoid malignancies in which of the number of CD19$^+$ B cells increased by 70–80% (Fig. 2*C*). The flow cytometry profile of 15 animals was not significantly different from that of wild-type mice.

In 80% of the animals spleen volumes were enlarged up to 30-fold in comparison with wild-type mice (Fig. 3*A*, *Left*). Histologically, extramedullary hematopoiesis was evident in almost all the examined animals (93%). Splenic benign lymphoproliferative disease (LPD) defined by abnormal B-cell–derived white pulp expansion with follicular structure disruption was observed in 49% of the animals.

A histological diagnosis of lymphoma was reached in 21 cases (28%) (Fig. 3*B*). A massive infiltration of extralymphoid tissues was evident in 18 animals, with lung being the most severely affected organ and kidneys being affected to a lesser degree (Fig. 3*C* and Fig. S2). Histological evidence of leukemia and bone

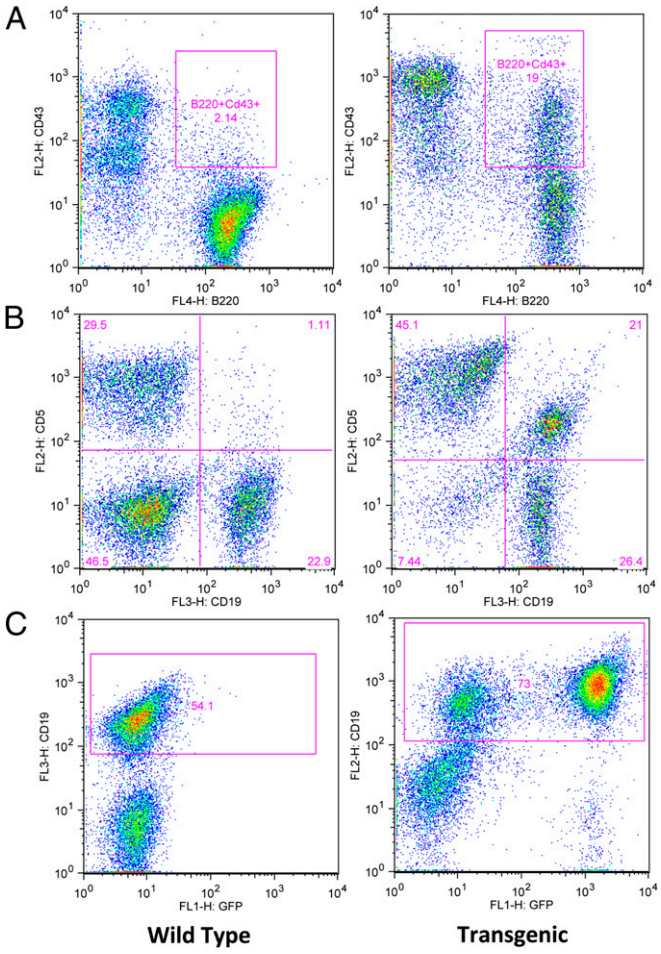

Fig. 2. Flow cytometry analysis of mice splenocytes for the three representative lymphoma phenotypes. (*A*) Significantly expanded B220$^+$ CD43$^+$ B1-stage B-cell population in the transgenic mice (19%) compared with wild-type mice (2.1%). (*B*) Significantly expanded CD19$^+$ CD5$^+$ B-CLL–like population in the transgenic mice (21%) compared with wild-type mice (2.5%). (*C*) Significantly expanded CD19$^+$ GFP$^+$ DLBCL-like population in the transgenic mice compared with wild-type mice.

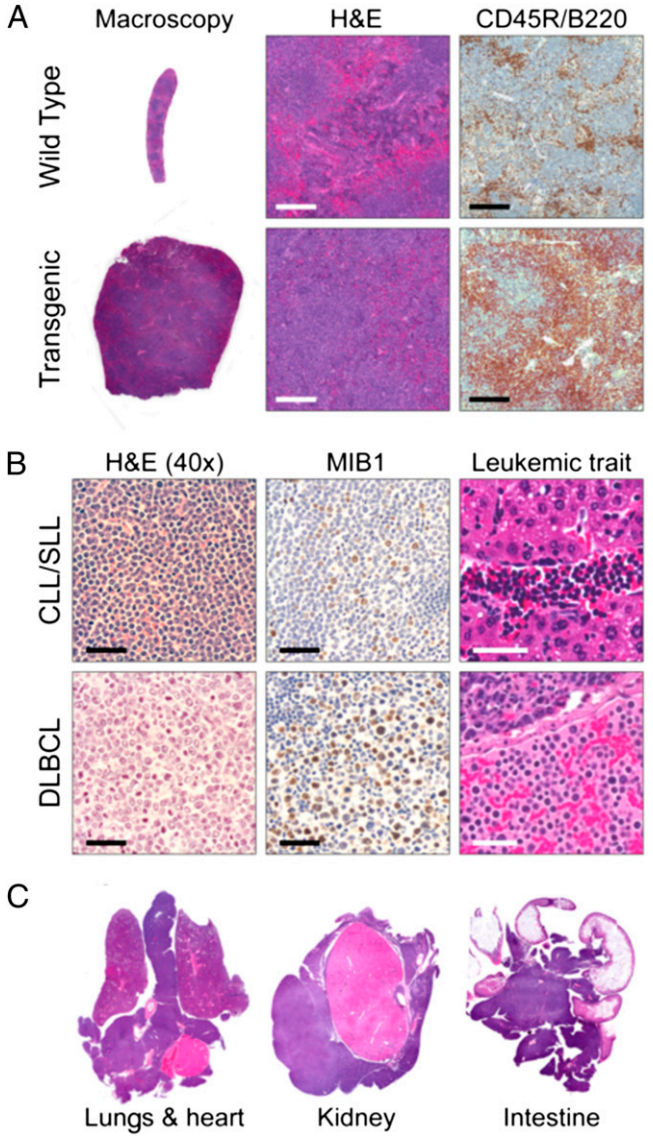

Fig. 3. (*A*) Histological analysis of spleen from wild-type and transgenic mice. (*Left*) Spleens from transgenic animals were enlarged up to 30-fold in volume compared with spleens from wild-type mice. (*Right*) Forty-nine percent of the transgenic mice presented an abnormal B-cell–derived white pulp expansion with follicular structure disruption, as demonstrated by B220 staining. (Scale bars, 1,000 μm.) (*B*) Representative phenotypes of CLL and DLBCL lymphomas observed in the Eu-17–92 mice. CLL presented as small-cell neoplasms characterized by a relatively low rate of mitoses and of the proliferation marker MIB1 immunoreaction (*Center*). DLBCL cases were characterized by large lymphocytes with vesicular nuclei and single prominent nucleoli, a high mitotic rate, and an MIB1 immunoreaction. Examples of leukemic cells within blood vessels are presented also. (Scale bars, 100 μm.) (*C*) Representative examples of extensive lymphoma infiltration of the mediastinum (*Left*), of the perirenal fat and renal capsule (*Center*), and of the mesenteric fat with involvement of the small intestine wall (*Right*).

marrow involvement was observed in 28% and 11% of the animals, respectively. In 13 cases, lymphomas were composed of relatively small lymphocytes, with heterochromatic nuclei and rare nucleoli, with a pattern similar to chronic lymphocytic leukemia/small lymphocytic leukemia (CLL/SLL). Discrete aggregates of small lymphocytes also could be observed in the peripheral blood smears. The other eight cases were characterized by large lymphocytes with vesicular nuclei and single prominent nucleoli, a high mitotic rate, and a "starry sky" appearance. These cases displayed a mature B-cell phenotype and were histologically reminiscent of the human DLBCL. No significant differences in lymphoproliferative lesions and lymphoma occurrence were observed among the three obtained *Eμ-miR-17~92* lines.

Molecular analysis of the rearranged IgV genes from five of 10 lymphoma samples confirmed their clonal derivation (Fig. S3). Cytogenetic analysis of lymphomatous B cells showed multiple structural rearrangements including inversion of chromosome 6, 41–42,XY,+15,+15[2][cp18]/40,XY[4], and Y,+2,inv (6)(B3G2), +15,+17,+18,+mar[20].

miRNome Analysis of Transgenic Mice B Cells and Splenocytes. The polycistronic *miR-17~92* cluster consists of six individual miRs, i.e., *miR-17* (5p and 3p), *miR-18*, *miR-19a*, *miR-20a*, *miR-19b-1*, and *miR-92a*. In addition, the paralogs of this cluster include *miR-106a~363* and *miR-106b~25*. Although, expression of the *miR-17~92* cluster was confirmed by qRT-PCR and Northern blotting, we also performed a global miR expression-profiling study to understand the overall miRnome and the internal miR circuits which may be induced/regulated by this cluster.

A total of 44 miRs (19 down- and 25 up-regulated) were differentially regulated in transgenic mice B cells compared with wild-type cells ($P < 0.05$) (Fig. 4 and Table S1). Ingenuity

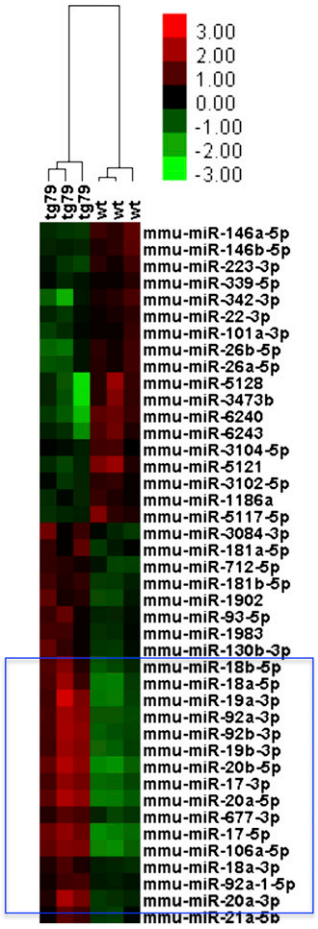

Fig. 4. 3D-Gene miR-expression profile of purified B cells from three transgenic and three wild-type mice. Rows represent individual genes; columns represent individual tissue samples. Pseudocolors indicate transcript levels below (green), equal to (black), or above (red) the mean. The scale represents the intensity of gene expression (log2 scale ranges from −3 to 3). The blue outline indicates the *miR-17~92* cluster.

Table 1. Expression of differentially regulated miRs paired with their target mRNAs using Ingenuity Pathway Analysis: up-regulated miRs and their targets that are down-regulated

Up-regulated miRs (and other miRs w/seed)	Down-regulated Target mRNA (inverse expression to miR)
miR-130a-3p (w/seed AGUGCAA)	**AAK1,** AIG1, ARL4A, **BACH2**, BBX, BPTF, BTG1, FBXO9, GADD45A, GADD45B, LIMD2, MBNL1, PDE4D, PHF17, PPARGC1A, RAPGEF4, **TAOK1,** TES, **TGFBR2, TRPS1, TSC22D1, TXNIP, ZNF148**
miR-17–5p, 20b-5p (w/seed AAAGUGC)	**AAK1,** ARL4A, BBX, CCNJ, CTSA, CYP4V2, DDX5, E2F5, ITPR2, ITSN2, JAK1, L3MBTL3, MIDN, NXF1, PHF1, PLEKHM1, PTP4A2, RAPGEF4, **RORA, RUNX1, SLAIN2, SLC16A6**, SLC16A9, **SMAD7,** SNRK, STK17B, **TAOK1, TGFBR2, TRPS1, TXNIP,** ZMYM2, **ZNF148**
miR-181a-5p (w/seed ACAUUCA)	**AAK1, BACH2,** BANK1, BPTF, CCNJ, CHD7, CPT1A, DDX5, DYRK2, E2F5, GDI1, KBTBD7, KMO, LPP, MBNL1, MPP5, RAB3IP, **RORA, RUNX1**, SAMD9L, **SLAIN2, SLC16A6**, SLC25A24, **SLC2A3, SMAD7, TAOK1, TGFBR2**, TREML4
miR-18a-5p (w/seed AAGGUGC)	**ABCC3**, BBX, CLCN4, CORO2A, DENND3, FBXO9, IL10RB, INADL, LPP, MBNL1, NKTR, PDE4D, **RORA, RUNX1, SATB1**, SH3BP2, SH3KBP1, SLC35D2, SNRK, **TAOK1**
miR-19b-3p, 19-a3p (w/seed GUGCAAA)	**AAK1, ABCC3,** ARHGAP5, BBX, BPTF, BTG1, CR2, EBF1, L3MBTL3, LIMD2, LPP, **MBNL1**, PHF17, RAB33B, RALGPS2, RAPGEF4, RNF167, **RORA, SATB1**, SH3KBP1, STAT2, **TAOK1, TGFBR2, TRPS1**, ZMYM2
miR-21–5p (w/seed AGCUUAU)	ANXA1, CHD7, FOXO1, HIST2H2BF, IL12A, **MBNL1,** RALGPS2, **SATB1, SMAD7, TGFBR2**
miR-3084–3p (w/seed UCUGCCA)	**AAK1,** ABCG1, ASB2, BCL6, BPTF, CCDC125, DAPP1, DUSP16, DYRK2, EIF2S3, GAB3, IL4R, KIAA1737, PAX5, RAB43, SIDT1, SLC7A14, **TSC22D1**, ZNF263
miR-92a-3p, 92b-3p (w/seed AUUGCAC)	**AAK1,** CD1D, DYRK2, KMO, LPP, P2RY13, PDE4D, PHF17, PLEKHM1, **RORA, SLC16A6, SLC2A3**, **SMAD7**, XPR1, **ZNF148,** ZNF287

Targets shared by multiple miRs are in bold and underlined.

Pathway Analysis (Ingenuity Systems, www.ingenuity.com) of differentially regulated miRs was paired with the mRNA expression data to decipher the key miR–mRNA networks, which may have role in cancer. We found that 20 miRs and 229 target mRNAs were dysregulated in our dataset: eight up-regulated miRs could be correlated with the 101 down-regulated target mRNAs, and 11 down-regulated miRs could be correlated with 66 up-regulated target mRNAs.

Further analysis of differentially regulated miRs driven by the ectopic *miR-17~92* overexpression and the predicted targets which had inverse expression provided some candidates which can be linked to B-cell lymphomas. Common targets shared by multiple miRs, which were in inverse correlation with the miR expression, are highlighted. In addition to the *miR-17~92* cluster, *miR-130a-3p*, *miR-181a-5p*, *miR-21–5p*, and *miR-3084–3p* were significantly overexpressed (Table 1). Among the miRs that were down-regulated in *Eμ-miR-17~92* B cells were *miR-101–3p*, *miR-146a-5p*, *miR-22–3p*, *miR-223–3p*, *miR-339–5p*, *miR-342–3p*, *miR-3473b*, *miR-26a-5p*, *miR-26b-5p*, *miR-330–3p/6240*, *miR-512–3p/1186a*, and *miR-702–5p/3102–5p* (Table 2).

We used the latest high-throughput miR-profiling technology from Firefly Bioworks to assay miR expression in fresh-frozen splenic tissues obtained from 67 mice (46 transgenic and 21 wild-type). In six animals, total RNA from purified splenic B cells also was available and was included in the analysis. The miR profile of the spleen was similar to that of purified B cells in all six observed cases, indicating that the spleen can be used safely to predict the miR profile without having to isolate splenic B cells (Fig. S4).

Hierarchical clustering of miR expression from the 67 mice shows three different classes according to miR-17~92 expression (Fig. 5). The class with the lowest miR-17~92 expression comprised most of the normal animals (95%); no mouse developed lymphoma, and LPD was observed in 14% of the mice. In the class with the middle level of miR-17~92 expression, the incidence of LPD was 56%, and the incidence of SLL/CLL lymphomas was 11%. All the aggressive B-cell lymphoma/leukemia cases clustered in the class with the highest *miR-17~92* expression, with 79% of these mice showing lymphomas and the remaining 21% demonstrating LPD.

Table 2. Expression of differentially regulated miRs paired with their target mRNAs using Ingenuity Pathway Analysis: down-regulated miRs and their targets that are up-regulated

Down-regulated miR (and other miRs w/seed)	Up-regulated target mRNA (inverse expression to miR)
miR-101–3p (w/seed ACAGUAC)	AP3S1 ATP5G2, CD86, DNAJC15, GALNT7, LPHN2, PSMA5, RSL1D1, SLC30A7, **SLCO3A1**
miR-146a-5p (w/seed GAGAACU)	**ARID3A**, CKAP4, MTDH, **SLCO3A1**
miR-22–3p (w/seed AGCUGCC)	C15orf39, C19orf70, CD3EAP, CLIC4, **DUSP10,** FKBP1A, HN1L, LGALS1, MGAT3, MRPL23, MTDH, PHF5A, RYR1, SLC7A7, TPI1, UNG
miR-223–3p (w/seed GUCAGUU)	**API5, DUSP10**, FUBP3, GALNT7, MRPS17, POLDIP2, PSMA5, **ST3GAL1**, VIM, VPS26B
miR-339–5p (w/seed CCCUGUC)	ACY1, CACNA2D2, CD3EAP, COQ7, F11R, HPSE, MGAT3, PARD6G, STK19, TPD52L2
miR-342–3p (w/seed CUCACAC)	**ADAM9**, COQ7, CYP51A1, H2AFJ, MTDH, **SLC5A1**, SSR1
miR-3473b (w/seed GGCUGGA)	AKAP1, LPHN2, MPHOSPH6, NDUFA11, PLA2G12A, PLP2, POLDIP2, RSL1D1, SERHL2, **SLCO3A1**, **ST3GAL1**
miR-26a-5p, 26b-5p (w/seed UCAAGUA)	**ADAM9**, **ARID3A**, C20orf24, GALNT7, LSM2, MTDH NIP7, RAB11A, SLC30A7, **SLC5A1**
miR-330–3p, 6240 (w/seed CAAAGCA)	**API5**, ARHGAP20, AZIN1, C1QBP, FKBP1A, GALNT7, GTF2H5, PSMA5, RND3, **SLCO3A1**, TWSG1, VPS26B
miR-512–3p, 1186a (w/seed AGUGCUG)	**ADAM9**, RND3, RSL1D1, **SLCO3A1**
miR-702–5p, 3102–5p (w/seed UGAGUGG)	GTF2H5, POLR2H, PPIL1, **SLC5A1**

Targets shared by multiple miRs are in bold and underlined.

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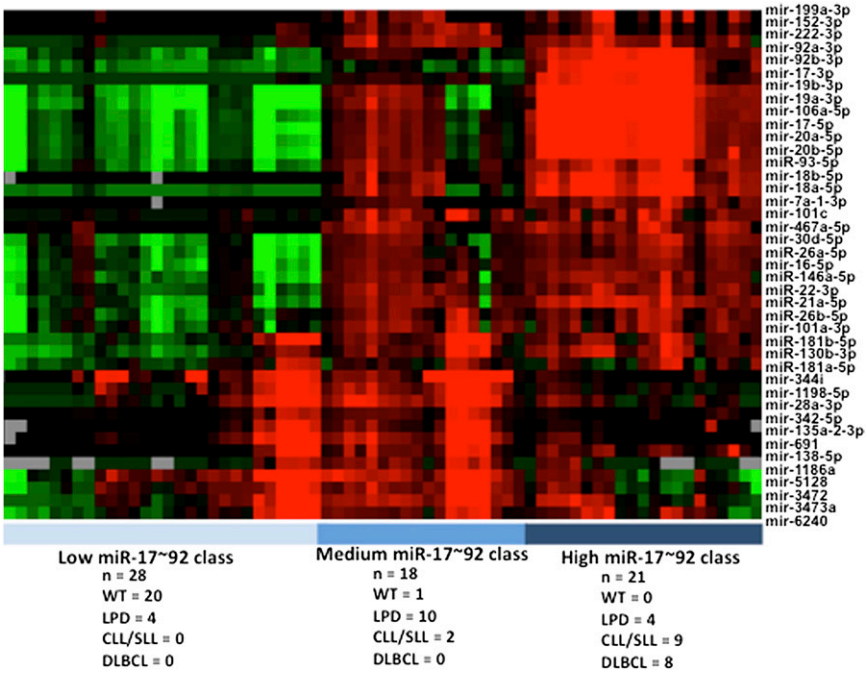

Fig. 5. Firefly miR-expression profile of 68 miRs in 46 transgenic and 21 wild-type mice showing that most aggressive B-cell lymphomas cluster with the highest *miR-17~92* expression class (*Right*). Wild-type mice clustered in the lowest *miR-17~92* class (*Left*). Rows represent individual genes; columns represent individual tissue samples. Pseudocolors indicate transcript levels below (green), equal to (black), or above (red) the mean. The scale represents the intensity of gene expression (log2 scale ranges from −3 to 3). The distribution of mice according to genotype and histopathologic analysis is shown also.

Functional and Pathways Analysis of eμ-miR-17~92 Transgenic Mice. mRNA-expression profiling of the purified B cells from the transgenic mice provided a global insight into the *miR-17~92*–mediated gene expression and how it contributes to lymphoma. We found that a total of 680 genes were differentially expressed in B cells from *eμ-miR-17~92* and wild-type mice ($P < 0.01$). Of these, 356 genes were down-regulated, and 324 were up-regulated (Dataset S2).

The top five pathways represented in the down-regulated gene set included T-helper cell differentiation, PI3K signaling in B lymphocytes, B-cell receptor signaling, growth arrest and DNA damage 45 (GADD45) signaling, and IL-4 signaling (Table 3). The pathways represented in the up-regulated gene set included mitochondrial dysfunction, purine nucleotides de novo biosynthesis II, integrin-linked kinase (ILK) signaling, GDP-L-fucose biosynthesis I, and cardiac β-adrenergic signaling (Table 4).

Table 3. Molecules represented in the top five canonical pathways that are down-regulated in B cells from eμ-miR-17~92 transgenic mice

Ingenuity canonical pathways	-log (*P* value)	Ratio	Molecules
T-helper cell differentiation	6.92E00	1.8E-01	STAT4, TGFBR2, IL18, IL4R, IL12A, IL21R, IL10RB, HLA-DOB, BCL6
PI3K signaling in B lymphocytes	2.58E00	6.31E-02	PTPRC, FYN, IL4R, PPP3CB, DAPP1, ITPR2, CR2
B-cell receptor signaling	2.55E00	5.59E-02	PAX5, PTPRC, PPP3CB, FOXO1, INPP5F, DAPP1, CD22, BCL6
GADD45 signaling	2.43E00	1.58E-01	GADD45B, GADD45A, GADD45G
IL-4 signaling	2.31E00	7.58E-02	IL4R, JAK1, INPP5F, HLA-DOB, FCER2

Discussion

Our study provides evidence of B-CLL development in mouse B cells overexpressing *miR-17~92*. The fact that a subset of TG mice develops B1-cell lymphomas and the others develop B-CLL further supports the model in which B1 cells are considered a precursor to B-CLL. This model along with the other animal models for B-CLL such as *Eμ-Tcl1* (10) and *Eμ-miR-29* (11) can greatly help elucidate the mechanism of this disease. Expression profiling of these lymphomas/leukemias provides an important insight into the oncogenic mechanisms triggered by this oncogenic

Table 4. Molecules represented in the top five canonical pathways that are up-regulated in B cells from eμ-miR-17~92 transgenic mice

Ingenuity canonical pathways	-log (*P* value)	Ratio	Molecules
Mitochondrial dysfunction	2.38E00	4.29E-02	NDUFV2, NDUFA11, TXN2, NDUFA7, ATP5G2, NDUFB10
Purine nucleotides de novo biosynthesis II	2.22E00	1.82E-01	ADSSL1, IMPDH2
ILK signaling	2.12E00	3.77E-02	ITGB1, MYC, RND3, VIM, KRT18, PPP1R14B
GDP-L-fucose biosynthesis I (from GDP-D-mannose)	1.67E00	5E-01	TSTA3
Cardiac β-adrenergic signaling	1.58E00	3.81E-02	PRKAR2B, PPP1R14B, APEX1, AKAP1

miR cluster. *MiR-17~92* has been linked to multiple cancers, and its expression signature was observed in various lymphomas, including anaplastic large cell lymphomas, by a recent study (12). A study by Fassina et al. (13) demonstrated the utility of the *miR-17~92* cluster as a diagnostic tool in large B-cell malignancies. Our study provides evidence supporting efforts to use the *miR-17~92* expression signature as a biomarker of B-cell lymphomas for therapeutic interventions.

Corroboration of mRNA and miR profiles provided useful networks that can be studied further to understand mechanisms of cancer pathogenesis. The down-regulated mRNAs in mice overexpressing *miR-17~92*, which also are the predicted targets of this cluster, provide a list of candidate genes that can be studied further in humans. For instance 17 genes—*AAK1, ABCC3, BACH2, MBNL1, RORA, RUNX1, SATB1, SLAIN2, SLC16A6, SLC2A3, SMAD7, TRPS1, TAOK1, TGFβR2, TXNIP, TSC22D1*, and *ZNF148*—are among the down-regulated genes in *Eμ-miR-17~92* B cells, which also are the predicted targets of *miR-17~92*. Except for *TGFβR2,* none of the other targets have been linked to *miR-17~92* overexpression, but there is evidence of their involvement in lymphomas. Li et al. (14) showed that *miR-17~92* targets *TGFβR2* to induce cell proliferation (14). *TAOK1* was found to be a fusion partner of *PAX5* in B-cell acute lymphoblastic leukemia (15). *Abcc3* is a member of the ABC transporter family and is involved in drug transport or redistribution of imatinib and dasatinib anticancer agents. It also was found to be overexpressed in the Hodgkin and Reed Sternberg cells in multiple cases of Hodgkin lymphoma (16). *Abcc3* is one of the multidrug resistance-associated proteins (MRP), and hence its expression in association with *miR-17~92* can be useful biomarker of drug resistance in cancer. Interestingly, *SLC16A6* and *SLC2A3* are among the few members of the solute carrier (SLC) transporter family, predicted to be targeted by multiple miRs (*miR-17–5p*/*20b-5p*, *miR-181a-5p*, *miR-181a-5p*, and *miR-92a-3p*/*92b-3p*) and down-regulated in *Eμ-miR-17~92* B cells. Still other SLC transporters down-regulated in B cells from *Eμ-miR-17~92* mice include *SLC25A24, SLC25A24*/*SCAMC-1, SLC35D2,* and *SLC7A14.* SLC transporters have been implicated in some solid malignancies; here we report the involvement of SLC transporters in B-CLLs. Exonic variants in *SLC16A6* were found to be strongly correlated with disease heritability in breast cancer (17). A recent genome-wide association study reported genetic variants in *SLC35D2* and their role in non-small cell lung cancer (18). Further investigation into the roles of different transporter gene families and their regulation in lymphomas can provide new avenues for drug discovery and biomarker development.

Among the seven common genes that were up-regulated predicted targets of the 11 miRs down-regulated in *Eμ-miR-17~92* B cells (*miR-101–3p*, *miR-146a-5p*, *miR-22–3p*, *miR-223–3p*, *miR-339–5p*, *miR-342–3p*, *miR-3473b*, *miR-26a-5p*/*26b-5p*, *miR-330–3p*/*6240*, *miR-512–3p*/*1186a*, and *miR-702–5p*/*3102–5p*) are drug transporters of the SLC family such as *SLCO3A1, SLC5A1; ARID3A, API5, DUSP10, ST3GAL1*, and *ADAM9*/*MDC9*. Further investigation into how *miR-17~92* regulates these transporters may provide useful insight into drug design, delivery, and resistance mechanisms. The heterogeneity of pathological lesions found in this model may relate to that seen in human lymphomas, and thus the model may help provide a better understanding of the molecular mechanisms that contribute to the development of these tumors.

Materials and Methods

Eμ-miR-17~92 transgenic mice were generated by cloning the mouse *miR-17~92* primary sequence into the BamHI and SalI sites of the plasmid containing a mouse V_H promoter (V186.2) and the Ig heavy-chain enhancer (*IgH-Eμ*) (19) along with the humanized renilla GFP and the SV40 poly(A) site at The Ohio State University Genetically Engineered Mouse Models Core. Overexpression was verified using qRT-PCR. A total of 104 mice (74 transgenic and 30 wild-type) were observed over a 2-y period for disease symptoms. They were assayed using flow cytometry and immunophenotyping to determine the B-cell development stages. The tissues were formalin fixed for histology and immunohistochemistry. Pan B cells were purified using a B-cell isolation kit (Miltenyi), and total RNA was isolated using TRIzol for miR and mRNA profiling. Expression profiling was done using the 3D-Gene mouse chip from Toray Industries. The data were analyzed using BRB array tools and Ingenuity Pathway Analysis.

Detailed methods are provided in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank Kay Huebner for critical reading of the manuscript; Nicola Zanesi for helpful scientific discussion; Dorothee Wernicke and Sharon Palko for administrative support; Anthony Fusco for Firefly data analysis; Lindsey Eickel, Aisha Nuur, Fata Nyei, Reid Neinast and Janae Dulaney for technical support; Daniel Lundy, Bryan McElwain, and Katrina Moore of the Flow Lab; Krista La Perle, Deborah Devor-Henneman, and her staff at the Comparative Pathology and Mouse Phenotyping Shared Resource (CPMPSR) for help in histological analysis; Lisa (Maysoon) Rawahneh histology; current and former ULAR staff and veterinarians for help in animal husbandry and well being; and Nyla Heerema and Jadwiga Zabrowski of the Cytogenetics Core Laboratory for karyotyping services and help. This work was supported by National Institutes of Health/National Cancer Institute Grant R01 CA124541 to C.M.C.

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