Review

The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease

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The miR-17/92 cluster is among the best-studied microRNA clusters. Interest in the cluster and its members has been increasing steadily and the number of publications has grown exponentially since its discovery with more than 1000 articles published in 2012 alone. Originally found to be involved in tumorigenesis, research work in recent years has uncovered unexpected roles for its members in a wide variety of settings that include normal development, immune diseases, cardiovascular diseases, neurodegenerative diseases and aging. In light of its ever-increasing importance and ever-widening regulatory roles, we review here the latest body of knowledge on the cluster’s involvement in health and disease as well as provide a novel perspective on the full spectrum of protein-coding and non-coding transcripts that are likely regulated by its members.

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Facts

- MiR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a-1 are members of the miR-17/92 cluster.
- The miR-17/92 cluster is important in cell cycle, proliferation, apoptosis and other pivoval processes.
- The miR-17/92 cluster is important in normal development and also the first group of microRNAs (miRNAs) to be implicated in a human syndrome (Feingold syndrome).
- The miR-17/92 cluster is also known as ‘oncomiR-1’.
- The miR-17/92 cluster is very often dysregulated in hematopoietic and solid cancers.
- The miR-17/92 cluster is often dysregulated in cardiovascular, immune and neurodegenerative diseases.
- The miR-17/92 cluster has been implicated in age-related conditions.
- There are two models of miRNA targeting: the ‘standard’ that has been in use for a decade and the ‘expanded’ that is emerging with the help of recent technological advances.
- The ‘standard’ model assumes Watson–Crick pairing in the seed region of a miRNA and targets that are primarily in the 3’ untranslated region (3’UTR) and conserved across genomes.
- The ‘expanded’ model also incorporates Watson–Crick pairing but additionally allows for combinations of unmatched bases and G:U wobbles in the ‘seed’ region; moreover, the targets can be anywhere along the messenger RNA (not just the 3’UTR) as well as in the intergenic and intronic genomic space; under this model, miRNA targets need not be conserved.

Open Questions

- What currently unsuspected processes and human diseases/conditions are regulated by the miR-17/92 cluster?
- Are there any protein-coding genes that are important for human diseases or conditions and are regulated by the miR-17/92 cluster?
- Does the miR-17/92 cluster have functionally significant genomic targets in the intergenic and intronic parts of the genome?
- Are there additional paralogues of the miR-17/92 cluster that have not yet been reported?
- The presence of guanines and thymines in the seed region of the cluster’s members suggests great potential for targeting under the ‘expanded’ model; what is the relative fraction of the cluster’s targets under the ‘expanded’ model?

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Abbreviations: 3’UTR, 3’ untranslated region; 5’UTR, 5’ untranslated region; CLIP-seq, crosslinking and immunoprecipitation followed by high-throughput sequencing; Ago, Argonaute protein; AD, Alzheimer’s disease; AML, acute myeloid leukemia; APC, Adenomatous Polyposis Coli; APP, amyloid protein precursor; ATM, activating transcription factor 4; C13orf25, chromosome 13 open reading frame 25; C. elegans, Caenorhabditis elegans; CAD, coronary artery diseases; CDS, coding sequence; CLL, chronic lymphocytic leukemia; CRC, colorectal cancer; CTGF, connective tissue growth factor; D. melanogaster, Drosophila melanogaster; ENCODE, the Encyclopedia of DNA Elements; ER, estrogen receptor; IBD, inflammatory bowel diseases; IRES, internal ribosome entry site; IFN-γ, interferon-γ; Isl-1, insulin gene enhancer protein; HC, hepatocellular carcinoma; HSC, hematopoietic stem cells; MAPK, mitogen-activated protein kinase; M-CSF, macrophage-colony stimulating factor; MIR17HG, the miR-17/92 cluster host gene (non-protein coding); miRNAs, microRNAs; MTF, microphthalmia-associated transcription factor; MLL, mixed-lineage leukemia; MS, multiple sclerosis; MSCV, murine stem cell virus; ncRNAs, non-coding RNAs; Nf2, nuclear factor-erythroid-2-related factor 2; Nts, nucleotides; PTEN, phosphatase and tensin homolog; STAT3, signal transducer and activator of transcription 3; Tbx1, T-box 1 protein; TFs, transcription factors; TNBC, triple negative breast cancer; TSP-1, thrombospondin-1; VHL, von Hippel-Lindau tumor suppressor

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MiRNAs are abundant non-coding RNAs (ncRNAs), ∼22 nucleotides (nts) in length, which have significant roles in regulating gene expression. The first animal miRNA, lin-4, was discovered during a genetic screen in Caenorhabditis elegans (C. elegans) and was found to repress the expression of the protein-coding gene lin-14. In 2000, a second miRNA, the well-conserved let-7, was discovered and functionally characterized as important for C. elegans development.

Since then, thousands of miRNAs have been predicted and identified in animals, plants and viruses (see http://www.mirbase.org).

Herein, we focus on the miR-17/92 cluster of miRNAs and review the current knowledge to date as to the roles of its members in health and disease. In light of recent findings, we also examine and discuss the topic of miRNA target identification in the context of the miR-17/92 cluster.

The Cluster and its Paralogues

In 2004, a novel gene, ‘chromosome 13 open reading frame 25’ or C13orf25 for short, was identified. Analysis of 70 human B-cell lymphoma cases showed amplification of this region. The miR-17/92 cluster as it is now known is located in the locus of the non-protein-coding gene MIR17HG (the miR-17/92 cluster host gene) (also known as C13orf25).

The miR-17/92 cluster transcript spans 800 nts out of MIR17HG’s 7 kb and comprises six miRNAs: miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a-1 (Figure 1). The miR-17/92 cluster is conserved among vertebrates. Soon after its discovery, the ectopic expression of a truncated version of the cluster (lacking miR-92) in B-cell lymphoma revealed its oncogenic character and miR-17/92 was given the distinction of being the first ‘oncomir’.

The human genome contains two paralogues of the main cluster (Figure 2): the miR-106b/25 and the miR-106a/363 cluster, respectively. MiR-106b/25 is located on chromosome 7 (7q22.1), in the 13th intron of the MCM7 gene. MiR-106a/363 is located on chromosome X (Xq26.2). The miR-106b/25 cluster comprises three miRNAs: miR-106b, miR-93 and miR-25 (Figure 2). The miR-106a/363 cluster comprises six miRNAs: miR-106a, miR-18b, miR-20b, miR-19b-2, miR-92a-2 and miR-363. MiR-17/92 and miR-106b/25 are expressed abundantly in a wide spectrum of tissues but miR-106a/363 is expressed at lower levels.

Together these three miRNA clusters represent a combined total of 15 miRNAs that form four “seed” families: the miR-17 family, the miR-18 family, the miR-19 family and the miR-92 family (Figure 3).

Transcriptional Regulation of the Cluster

One of the early findings was C-MYC’s involvement in activating MIR17HG transcription through a site that is located 1484 nts upstream of MIR17HG’s transcription start site. N-MYC also transcriptionally activates MIR17HG as well as

Figure 1: Genomic representation of the human miR-17-92 cluster host gene (MIR17HG) and neighborhood genes on Chr 13q31.1-q33.1. (a) Genomic representation of genes located ± 10 kb around human MIR17HG. (b) Genomic representation of MIR17HG. Two transcripts are shown in light blue and individual members of the cluster represented as red rectangles. The two panels were created using the UCSC genome browser (http://genome.ucsc.edu/)
E2F1 and E2F3. The data show close functional interactions between c-Myc/n-Myc and the miR-17/92 cluster. Both c-Myc and n-Myc can directly bind to the promoter of miR-17/92 and initiate transcription. Indeed, some patients with an c-Myc and n-Myc can directly bind to the promoter of miR-17/92 and initiate transcription.17,21,22

Figure 2 Members of the miR-17/92 cluster and its two paralogues miR-106a/363 and miR-106b/25 and their chromosomal location. Red: members of the miR-17 family; blue: members of the miR-18 family; green: members of the miR-19 family; orange: members of the miR-92 family

Figure 3 Sequences of the members of the miR-17/92 cluster (in bold face) and its two paralogues miR-106a/363 and miR-106b/25. The sequences are divided into four families according to the miRNA ‘seed’ (the sequence spanning positions 2 through 7 inclusive counting from the 5′ end of the miRNA). The ‘seed’ in each case is shown in boldface and is highlighted in blue

Main Targets of the miR-17/92 Cluster

Phosphatase and tensin homolog (PTEN) and E2Fs were among the first validated miR-17/92 targets.15,17,19 Reporter assays revealed targets for miR-19a and miR-19b-1 in PTEN's 3'UTR, and the introduction of miR-19a and miR-19b-1, or of the full cluster, in miR-17/92-deficient cells sufficed to restore PTEN expression levels.15 In addition, miR-17 and miR-20a modulate the expression of E2F1,17,19

Lastly, miR-20a targets the 3'UTRs of both E2F2 and E2F3 (Figure 4).19

The ability of the cluster's members to cooperate is evident in the context of TGF-β signaling. In particular, miR-17 and miR-20a directly target the TGF-β receptor II (TGFRBII), whereas miR-18a targets Smad2 and Smad4, two members of the TGF-β signaling pathway.35–37 TGF-β activation exerts an effect mediated in part by the cyclin-dependent kinase inhibitor (p21) and the apoptosis facilitator BCL2L11 (BIM), both of which are targeted by miR-17/92.35,38 In addition, BCL2L11 is targeted by miR-20a, miR-92, miR-19a and miR-19b-1 and also by miR-106b/25.39 During the endoplasmic reticulum related stress, unfolded protein response TFs, activating TFs, activating transcription factor 4 (Aft4) and nuclear factor-erythroid-2-related factor 2 (Nrf2) are activated and downregulate Mcm7, the host gene for the miR-106a/25 cluster. Downregulation of miR-106b/25 and repression of BCL2L11 consequently trigger apoptosis.39

Lastly, miR-18a and miR-19 directly repress the anti-angiogenic factors thrombospondin-1 (TSP-1) and connective...
tissue growth factor (CTGF). In addition, miR-17 and miR-20a participate in the regulation of the insulin gene enhancer protein (Isl-1) and the T-box 1 protein (Tbx1) (Figure 4).

**MiR-17/92 and Normal Development**

The miR-17/92 cluster is highly expressed in embryonic cells and has an important role in development. MiR-17/92 was the first group of miRNAs to be implicated in a developmental syndrome in humans. Indeed, studies of patients with Feingold syndrome revealed an important role for the miR-17/92 cluster in normal skeletal development. Human patients with heterozygous microdeletions in the MIR17HG locus have autosomal dominant Feingold syndrome, characterized by multiple skeletal abnormalities in the fingers and toes, short stature and microcephaly. Some patients also show various degrees of learning and developmental disabilities.

Subsequent mouse studies showed that deletion of the miR-17/92 cluster is perinatal lethal. MiR-17/92−/− embryos exhibit severe skeletal abnormalities and recapitulate the phenotype observed in patients with Feingold syndrome. The mice are also smaller in size than normal embryos, and die at birth from cardiac defects and lung hypoplasia. The miR-17/92 cluster is involved also in normal lung morphogenesis, epithelial proliferation and branching through the targeting of signal transducer and activator of transcription 3 (STAT3) and mitogen-activated protein kinase 14 (MAPK14). The overexpression of the miR-17/92 cluster leads to lung epithelium hyper-proliferation and suggests a role in lung cancer.

Analogously to the miR-17/92 studies that indicated a role in B-cell differentiation, the normal process of B-cell maturation in miR-17/92−/− mice is blocked during the progression from pro-B to pre-B cells. Mice with a deleted miR-17/92 cluster have a reduced number of pre-B cells at E18.5. In experiments with adult mice whose hematopoietic system is reconstituted with fetal liver cells from a miR-17/92Δneo/Δneo embryo at E14.5, the number of circulating lymphocytes, circulating B cells, splenic B cells and pre-B cells bone marrow cells is significantly reduced compared with mice with reconstituted fetal liver cells from wild-type embryos at 8–10 weeks post transplant.
stem cells (HSC) derived from fetal liver of Eμ-myc transgenic mice expressing miR-17/19 under the control of murine stem cell virus (MSCV) show a massive enlargement of lymph nodes, splenic hyperplasia, infiltration of the thymus by lymphoma cells and leukemias. Moreover, almost half of the animals in the test group exhibited hind leg paralysis as the result of tumors at the lumbar node. These results suggest the importance of miR-17/92 in normal B-cell development and survival. On the other hand, overexpression of the cluster was also shown to cause lymphoproliferative diseases.

Parallel studies have also implicated miR-17/92 in normal lymphocyte development. In mouse knockout models, miR-19b-1 and miR-17 were shown to promote T-cell expansion; the mice display reduced lymphocyte proliferation that was attributed to the promotion of interferon-γ (IFN-γ) production by miR-19b-1 and the promotion of a Th1 response by miR-17 and miR-19b-1. Indeed, miR-17 and miR-19b-1 have an important role in promoting B-cell proliferation, protecting B-cells from death, supporting IFN-γ production and suppressing T-cell differentiation.

The Cluster as an Oncogene

We next review the increasing body of literature on the cluster’s oncogenic role (Table 1).

### Table 1: Relative expression of individual miRNAs from the miR-17/92 cluster or its paralogues in normal development, cancer, other diseases and age-related conditions

| MiRNA   | Normal skeletal formation/endothelial cells | Long-term development | B-cell development | T-cell lymphoma | B-cell ALL | MLL leukaemia/AML (11;19) | T-cell lymphoma | Melanoma | Retinoblastoma | Colorectal cancer | Head and neck cancer | Pancreatic cancer | Breast cancer | ovarian cancer | Lung cancer | Renal cancer | Hepatocellular carcinoma | Osteosarcoma | Gastric cancer | Nephroblastoma | Urothelial cancer | Cardiovascular disease | Alzheimer’s disease | Multiple sclerosis | Ageing |
|---------|------------------------------------------|-----------------------|--------------------|----------------|------------|----------------------------|----------------|-----------|----------------|-----------------|---------------------|-------------------|----------------|----------------|-------------|-----------|----------------------------|-------------|----------------|----------------|------------------|---------------------|-------------------|---------------------|--------|
| miR-17  | ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| miR-18a | ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| miR-19a | ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| miR-20a | ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| miR-19b-1 | ? ? ? ? ? ? ? ? ? ? ? ? ? ? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| miR-92a-1 | ? ? ? ? ? ? ? ? ? ? ? ? ? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| miR-106a | ? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| miR-106b | ? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| miR-18b | ? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| miR-93 | ? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| miR-25 | ? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

Notation: ?: expression of miRNAs is important for normal development; ??: over-expressed miRNAs; ???: under-expressed miRNAs; blue boxes: normal development; burgundy boxes: hematopoietic cancers; green boxes: solid cancers; violet boxes: other diseases; orange boxes: age-related conditions. Gray cells indicate inconclusive evidence or unavailable data.

B-cell Lymphomas

The miR-17/92 cluster was initially found amplified in diffuse cell lymphomas. Later, in B-cell lymphoma, an ectopically overexpressed truncated version that lacked miR-92 showed the cluster’s role as an oncogene. Moreover, as already discussed, c-Myc was shown to transcribe the truncated cluster in mouse models of B-cell lymphoma. These findings represent early evidence that miR-17/92 can act as an oncogene by suppressing apoptosis. MiR-18a levels in diffuse large B-cell lymphoma correlate strongly and negatively with survival (higher expression—shorter survival). As mentioned already, miR-19a and miR-19b-1 are necessary and sufficient to promote tumorigenesis B-cell lymphoma. In addition, the conditional knockout of miR-17/92 in Myc-driven lymphomas was shown to increase apoptosis and to reduce tumorigenicity and tumor progression.

B-cell Chronic Lymphocytic Leukemia

MiR-20a was found to correlate with diagnosis to treatment time in B-cell chronic lymphocytic leukemia (CLL) and thus can potentially serve as a blood biomarker. The cluster members miR-17 and miR-19b-1 are highly overexpressed in CLL cultures with fibroblast expressing human CD40 ligand (CD154) with IL-4. Another study found miR-20, miR-18a, miR-
19a and miR-92a to be overexpressed in CLL cultures, but at much lower levels than those of miR-17 and miR-19b-1.53

Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is a heterogeneous group of diseases with different genetic rearrangements, different prognosis and required treatment options. MiR-17-5p, miR-17-3p, miR-20a and miR-92 are upregulated in myeloid/lymphoid, or mixed-lineage leukemia (MLL), display rearrangements in AML and are downregulated in AML with the translocation t(8;21). On the other hand, miR-17-5p and miR-20a are downregulated in t(15;17). AML with MLL-rearrangements is considered to have intermediate or poor prognosis; moreover, it requires a different treatment from AML with t(8;21)/t(15;17) that usually carries a favorable prognosis.54 In mouse studies, the levels of miR-106 were found to be upregulated in AML and to target Sequestosome 1 (SQSTM1).55 In AML characterized by the translocation t(8;16)(p11;p13), miR-17/92 is downregulated.56 However, there is no significant difference in the expression of MYC between t(8;16)(p11;p13) AML and other types of AML suggesting that other mechanisms of downregulation, for example, methylation, may be at work.56

T-cell Lymphoma

In an experiment with the SL3-3 murine leukemia virus, 2545 BALB/c newborn mice were infected and nearly all developed T-cell lymphoma. Quantitative RT-PCR analysis showed elevated expression of miR-17/92 after virus integration.57 The miR-17/92 integration sites were found to cluster together at three distinct regions; the integration sites within each such region were ~1 kb apart.57

Retinoblastoma

Overexpression and genomic amplification of miR-17/92 were shown in retinoblastoma.58 In particular, in Rb−/− and p107−/− retinoblastomas, ectopic expression of miR-17/92 induces rapid proliferation and disease onset. This increase in proliferation is linked to the miR-17 sub-family, which target the cell cycle inhibitors p24Gip1 and p57Kip2.58,59

Colorectal Cancer

MiR-17/92 was also found overexpressed in colon cancer.40 In a tumor engraftment model, upregulation of the cluster by Myc in colonocytes increased tumorigenesis by promoting angiogenesis through direct repression of TSP-1 and CTGF by miR-18a and miR-19, respectively.40 MiR-18a and miR-20a are significantly overexpressed in colorectal cancer (CRC); in fact, miR-18a is a marker of poor prognosis.50 In addition, miR-92 levels in colon adenocarcinoma have been shown to correlate negatively with BCL2L11 expression and, thus, with reduced apoptosis.61 High miR-17 expression correlates with low overall survival in patients with CRC.62 Another study that comprised 90 patients with CRC, 90 patients with inflammatory bowel diseases (IBD), 20 patients with gastric cancer and 50 healthy controls also confirmed high expression level of miR-17 and miR-92 in tumors and serum from patients with CRC.63 Patients with CRC had higher miR-92 levels compared with healthy controls or patients with IBD or gastric cancer.63 Interestingly, results revealed a correlation between miR-18a expression and Adenomatous Polyposis Coli (APC) mutation in CRC samples.64

Head and Neck Cancers

MiR-17/92 is often overexpressed in medulloblastomas, especially those with an active Sonic hedgehog signaling pathway. The cluster is overexpressed in mouse models in cerebellar granule neuron progenitors, where the tumor arises.65,66 Ectopic expression of miR-17/92 increases tumor formation through the suppression of TGF-β signaling upon orthotopic transplantation into immunocompromised mice.65,66 These studies suggest a tissue-specific function for members of the miR-17/92 cluster. The miR-17/92 cluster amplification was also reported in neuroblastomas and is linked to poor prognosis.67 Lastly, miR-17 has been shown to promote the growth of neuroblastoma cell lines.68

Pancreatic Cancer

In pancreatic cancer, miR-17, miR-18a, miR-19a and miR-19b-1 expression levels are increased.69,70 Another study also showed that the level of miR-18a in the blood is significantly higher before surgery in patients with pancreatic cancer compared with after surgery, suggesting the possibility that blood levels of miR-18 can potentially be used as a biomarker.70

Breast Cancer

Deep sequencing of triple negative breast cancer (TNBC) samples revealed a threefold increase of miR-17/92 levels.71 In estrogen receptor (ER)-positive breast cancer, it was shown that miR-18a/18b directly target the 3’UTR of the ERα.72 In addition, miR-17 and miR-20 are overexpressed in metastatic breast cancer73 and have been shown to directly suppress the 3’UTR of IL-8 and to inhibit cytokeratin 8 through cyclin D1.73 Another study has shown that miR-106b positively correlates with hometic TF Six1 expression levels in breast cancer.74 Six1 depends on the upregulation of the TGF-β pathway to induce epithelial–mesenchymal transition. In addition, high levels of miR-106b are indicative of shorter time to relapse.74

Ovarian Cancer

Studies have implicated the overexpression of miR-20a in proliferation and invasion in the OVCAR3 cell line, whereas the downregulation of miR-20a has been shown to lead to the suppression of proliferation and invasion. A possible mechanism is through binding to the amyloid protein precursor (APP), a gene of central importance in Alzheimer’s disease (AD).75
Lung Cancer
In lung cancer, miR-17-5p and miR-20a are overexpressed.76 Their targets include HIF-1α, PTEN, BCL2L11, CDKNA and TSP-1.76 A study of 221 lung cancer patients and 54 matching controls showed a significant increase of miR-17-5p expression in tumor and serum and a negative correlation with patient survival.77 However, the blood of non-small cell lung cancer patients had a low level of miR-17-5p.78

Renal Cancer
The miR-17/92 cluster is regulated by the von Hippel-Lindau (VHL) tumor suppressor: in the absence of VHL, miR-17/92 levels increase.79 Other studies have shown miR-17, miR-18a and miR-20a to be overexpressed in renal cancer; however, overexpression of these miRNAs did not correlate with survival.80,81

Hepatocellular Carcinoma
All six members of the miR-17/92 cluster are often overexpressed in hepatocellular carcinoma (HC).82 The use of antisense nts specific to all six members of the miR-17/92 cluster caused a 50% reduction in proliferation and anchorage-independent growth.82 In addition, several members of miR-17/26 and its paralogues (miR-92,83 miR-18a, miR-106b, miR-93 and miR-2584) are highly expressed in HC cells compared with paired non-tumor samples. Another study showed that miR-18, and the miR-106b/25 paralogue, was overexpressed in 50% of clinical samples used in the study.84 Cell culture studies have also shown that the knockdown of miR-106b/25 leads to decreased cell proliferation and anchorage-independent growth in three different cell lines: HepG2, HeLa and HuH7.84

Osteosarcoma
MIr-17/92 is overexpressed in osteosarcoma as demonstrated by luciferase assays.85 In particular, miR-17 and miR-20a are overexpressed in metastasized cells compared with parental cells.86 In addition, mouse studies with anti-miR-20a showed significant increase in lung metastases, possibly through the repression of FasL in the lung tissues.86

The Cluster in Other Diseases
Beyond cancer, the miR-17/92 cluster has been shown to have important roles in other human conditions including immune, cardiovascular and neurodegenerative diseases.87–90

Immune Diseases
The miR-17/92 cluster has a role in the innate and acquired immune response.88 In human cord blood, CD34 + hematopoietic progenitor cells differentiate into monocytes upon exposure to macrophage-colony stimulating factor (M-CSF) and the miR-17/92 cluster.91 MiR-17/92 also has a role in the acquired immune response.88 Another study has shown that autoimmunity, characterized by increased proliferation and survival of CD4 + T cell, could be caused by overexpression of the miR-17/92 cluster in the DN1 stage.88

Cardiovascular Diseases
MiR-92a is highly expressed in endothelial cells but overexpression of miR-92a in those cells under ischemic conditions was shown to inhibit angiogenesis.92 In a mouse model with leg ischemia, the administration of antagoniR-92a led to inhibition of miR-92a with consequent growth of new blood vessels and recovery from ischemia.93 In another study, endothelial cells from patients with coronary artery disease (CAD) exhibited higher levels of miR-17 and miR-92a compared with endothelial cells from healthy controls.93,94 In another study, the miRNA profile of patients with acute coronary syndrome showed an increase of miR-19 compared with patients with CAD.95 And a study of miRNA levels measured by quantitative RT-PCR in whole blood and serum showed reduced miR-19a levels in patients with CAD compared with healthy controls.

Neurodegenerative Diseases
The amyloid precursor protein APP generates the amyloid-β, Aβ, peptide through the ‘amyloidogenic’ pathway with the help of β- and γ-secretases. Aβ accumulates in extracellular spaces forming Aβ plaques. Members of the miR-17 family (i.e., miR-17, miR-20a, miR-106a and miR-106b) were shown to directly suppress APP in vitro.96,97 In the AD brain, miR-106b was shown to be downregulated in vivo.97 In relapsing patients with multiple sclerosis (MS), miR-18 was found to be overexpressed compared with controls.98 Another study of CD4 + T cells and B cells of relapsing and remitting MS patients shows the upregulation of miR-17-5p in CD4 + T cells and downregulation of miR-92 in B cells.99 Another study has shown under-expression of miR-17 and miR-20a in whole-blood samples from 59 MS patients compared with 37 healthy controls.100 The MS patients represented different disease types (primary progressive, secondary progressive and remitting-relapsing) and for the last 3 months before the study had not received any treatment. In addition, miR-106b and miR-25 were found to be upregulated in 12 relapsing-remitting MS patients and in 14 healthy controls.101 A recent analysis used an integrative approach to study miRNAs dysregulated in MS revealed that miR-20a and miR-20b target ~500 genes each.102 On a related note, profiling of miRNA expression in the brain of zebrafish (Danio rerio) showed that miR-92 is expressed in periventricular cells and in proliferative zones of larva and adult brain and down-regulated in mature neurons.103

The Cluster and Age-Related Conditions
Considering the importance of miR-17/92 in tumorigenesis, it was not long before the relation between dysregulation of these miRNAs and aging was discovered.104,105 Studies of different tissue types representing aging revealed down-regulation of miR-17, miR-92a, miR-20a and miR-106a.106 This suggests yet another role for these miRNAs, one that transcends cell cycle regulation and tumorigenesis. However, the mechanistic connection between downregulation of the cluster’s members and aging has yet to be elucidated.105
The miRNA Target Prediction Problem

The miRNA target prediction problem relates to the observation of conservation of miRNA sequences across animals and plants (and viruses). At the sequence level, most miRNAs are evolutionarily conserved among distant species. However, not every known miRNA is conserved: indeed, there are reports of miRNAs that are species- or genus-specific. Moreover, it is known that conserved miRNAs do not have the same functional behavior in different species: let-7, which is conserved between C. elegans and Drosophila melanogaster, is a characteristic example. In the worm, let-7 is a component of the heterochronic pathway; it is expressed at a late stage of larva development, regulates the transition from larva to adult and is embryonic lethal. In the fruit fly, let-7 knockout flies are externally normal but exhibit behavioral defects and juvenile features in their neuromusculature.

In recent work, we used molecular dynamics to analyze the crystal structure of the Argonaute (Ago) silencing complex and demonstrated in a very general way the existence of many admissible targets that transcend the ‘standard’ model that has been in use for more than a decade already. The molecular dynamics findings were further corroborated by publicly available Ago-immunoprecipitated and sequenced miRNA targets. These results provide strong evidence in support of an expanded model of miRNA targeting and are very relevant for the members of the miR-17/92 cluster and for our estimates of this cluster’s targetome (Figure 5). Glimpses of evidence supporting the ‘expanded’ model were also observed experimentally in earlier work as well as discussed in the literature.

The members of miR-17/92 clusters are ideal miRNAs for which to explore ‘unexpected targets’ under the ‘expanded’ model. Indeed, as all of the members have at least two G/U bases in their seed region, (Figure 3) they could potentially base pair with U and G, respectively, on the side of the target to create wobbles and additional targets (Figure 5). In addition, potential incorporation of bulges on either the miRNA or the target side would lead to an even higher number of non-standard targets.

There is also increasing evidence with regard to non-protein-coding transcripts that could be targeted by miRNAs. The evidence comes both from studies of individual miRNA:target pairs as well as global analyses. In particular, it has already been shown for several miRNAs, some belonging to the miR-17/92 cluster, that they target and suppress the expression of PTENP1, the PTEN pseudogene. The same study also showed targeting and regulation by miRNAs of the KRAS pseudogene KRASP1 as well as of several of the pseudogenes of OCT4. More recently, it was shown that miR-133 and miR-135 target a long non-coding RNA, linc-MD1, thereby regulating the expression of MAML1 and MEF2C, two TFs that activate muscle-specific gene expression. In addition, the advent of the crosslinking and immunoprecipitation followed by high-throughput sequencing (CLIP-seq) technology has enabled global studies of miRNA targeting preferences in a variety of contexts. Initial analyses of the available data have provided additional evidence that miRNA-targeted transcripts include numerous transcripts that are not protein-coding.

These findings indicate that the miR-17/92 targetome may be larger and further ranging than originally anticipated. As we saw above, several members of the miR-17/92 cluster have
been implicated in the regulation of non-protein-coding transcripts. In addition, the sequence composition of the seed region of the cluster’s members and in particular the presence of G/U’s in the seed provides them with an expanded base-pairing ability. Consequently, there is great potential that a very rich set of currently unrecognized heteroduplexes comprising miRNAs of the miR-17/92 cluster awaits discovery.

**Conclusion**

miR-17/92 is one of the best-known miRNA clusters. The cluster’s members have pivotal roles in normal development, and dysregulation of their expression leads to a wide array of diseases including hematopoietic and solid cancers, and immune, neurodegenerative and cardiovascular diseases. The cluster is also important because its members are the first described in the context of a developmental syndrome in humans. Related to this, other recent work uncovered novel important connections between the miR-17/92 cluster and aging.

Despite great progress in understanding the cluster’s roles, several key questions remain unanswered. For example, until the recently reported findings by the ENCODE project very little was known about the transcriptional control of the cluster by TFs as well as about the targeting of TFs by members of the cluster and its paralogues. Considering the ENCODE project’s findings, it is reasonable to conjecture that the actual transcription control of the cluster is significantly more complex than research to date has managed to reveal.

A parallel and very important question is that of elucidating the cluster’s targetome. The currently known set of validated protein-coding targets is small. In light of the many miRNAs that the cluster and its paralogues comprise and the recent evidence obtained through molecular dynamics studies and CLIP-seq data analyses, it is increasingly apparent that the true spectrum of targets is potentially very large. Additional research effort will be needed before the full complement of the cluster’s targets can be elucidated. It is also important to note that an increased target set opens up new opportunities and new avenues for therapeutic intervention in those settings, where one or more of the cluster’s members are dysregulated.

**Conflict of Interest**

The authors declare no conflict of interest.

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1. Bartel DP. Micrornas: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281–297.
2. Bartel DP. Micrornas: target recognition and regulatory functions. Cell 2009; 136: 215–233.
3. Ambros V. A hierarchy of regulatory genes controls a larva-to-adult developmental switch in Caenorhabditis elegans. Cell 1989; 57: 43–57.
4. Ruvkun G, Giusto J. The Caenorhabditis elegans heterochronic gene lin-14 encodes a nuclear protein that forms a temporal developmental switch. Nature 1989; 338: 313–319.
5. Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettendorf M, Ruvkun G, Shiekhattar R et al. The 21-nt small-interfering RNA pathway is used for both gene silencing and transgene activation in C. elegans. Cell 2000; 101: 25–36.
6. Chang S, Tagawa H, Kamin S, Tsuzuki S, Karpas A, Kira S et al. Identification and characterization of a novel gene, c10orf25, as a target for 13q31-32 amplification in malignant lymphoma. Cancer Res 2004; 64: 3087–3095.
7. Mendell JT. Mirrored roles for the mir-17-92 cluster in development and disease. Cell 2008; 133: 217–222.
8. Diosdado B, van de Wiel MA, Terhaar Sive Droste JS, Mongera S, Postma C, Meijerink APW et al. Genotype-tumor interaction analysis for oncogenic micrornas in neuroblastoma. Mol Cell Biol 2010; 30: 8233–8246.
9. Ota A, Tagawa H, Kamin S, Tsuzuki S, Karpas A, Kira S et al. Identification and characterization of a novel gene, c10orf25, as a target for 13q31-32 amplification in malignant lymphoma. Cancer Res 2004; 64: 3087–3095.
10. Sylvestre Y, De Guire V, Querido E, Mukhopadhyay UK, Bourdeau V, Major F et al. A miR-17/92 cluster of micrornas in human hematological malignancies. J Exp Med 2009; 206: 2135–2143.
11. Diosdado B, van de Wiel MA, Terhaar Sive Droste JS, Mongera S, Postma C, Meijerink APW et al. Repression of the mir-17-92 cluster promotes proliferation and inhibits differentiation of lung cancer cells. Int J Cancer 2008; 122: 699–704.
12. Sarboui H, Arthaud S, Gudelis J, Sruoga N, Ciofini G et al. B-cell lymphoma microRNA signature targeting ret proto-oncogene. Leukemia 2012; 26: 1113–1116.
13. Kim K, Chadalapaka R, Lee SO, Yamada D, Sastre-Garau X, Defossez PA et al. Identification of oncogenic micrornas-17-92/bTCbl2/spcility protein alpha in breast cancer. Oncogene 2012; 23: 4657–4665.
14. Xiao C, Sinivasan L, Calado DP, Patterson HC, Zhang B, Wang J et al. Lymphoproliferative disease and autoimmunity in mice with increased mir-17-92 expression in lymphocytes. Nat Immunol 2008; 9: 405–414.
15. Selvaraj A, Anwar M, Shahrokh A et al. A miR-17/92 cluster of oncogenic micrornas in myc-induced b-cell lymphomas. Proc Natl Acad Sci USA 2010; 107: 1553–1558.
16. Wang J, Rice E, Cao X, Wang M, Martin J, Frey S et al. Disentangling Perk-dependent and -independent functions of oncogenic miRNAs in myc-induced b-cell lymphomas. Mol Cell 2010; 39: 657–667.
17. Wang J, Rice E, Cao X, Wang M, Martin J, Frey S et al. A miR-17/92 cluster of oncogenic micrornas in b-cell lymphoma. Proc Natl Acad Sci USA 2010; 107: 1553–1558.
116. Easow G, Telemann AA, Cohen SM. Isolation of mirorna targets by mirrn and immunopurification. RNA 2007; 13: 1198–1204.
117. Baek D, Villem J, Shin C, Camargo FD, Gyggi SP, Bartel DP. The impact of mirrnas on protein output. Nature 2008; 455: 64–71.
118. Selbach M, Schwannhauser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by mirrnas. Nature 2008; 455: 58–63.
119. Chi SW, Zang JB, Mele A, Darnell JR. Argonaute hits-clip decodes mirrna-mrn interaction maps. Nature 2009; 460: 479–486.
120. Fabian MR, Sonenberg N, Filipowicz W. Regulation of mrna translation and stability by mirrnas. Annu Rev Biochem 2010; 79: 351–379.
121. Hafner M, Landthaler M, Burger L, Haffner M, Bierne J, Bemmir P et al. Trancriptome-wide identification of ma-binding protein and mirrna target sites by par-clip. Cell 2010; 141: 129–141.
122. Thomas M, Lieberman J, Lai A. Desperately seeking mirrna targets. Nat Struct Mol Biol 2010; 17: 1169–1174.
123. Zicouls DG, Lovci MT, Hutt KR, Liang SY, Pasquinelli AE et al. Comprehensive discovery of endogenous argonaute binding sites in Caenorhabditis elegans. Nat Struct Mol Biol 2010; 17: 173–179.
124. Chi SW, Hannon GJ, Darnell JR. An alternative mode of mirrna target recognition. Nat Struct Mol Biol 2012; 19: 321–327.
125. Skalsky RL, Corcoran DL, Goettner E, Frank CL, Kang D, Haffner M et al. The viral and cellular mirrna targetome in lymphoblastoid cell lines. PLoS Pathogens 2012; 8: e1002484.
126. Miranda KC, Huyrth T, Taylor Y, Ang YS, Tam W, Thomson AM et al. A pattern-based method for the identification of mirrna binding sites and their corresponding heteroduplexes. Cell 2008; 136: 1203–1217.
127. Forman JJ, Legesse-Miller A, Coller HA. A search for conserved sequences in coding regions reveals that the let-7 mirrna targets dicer within its coding sequence. Proc Natl Acad Sci USA 2008; 105: 14879–14884.
128. Kloosterman WP, Wetholds E, Ketting RF, Plasterk RH. Substrate requirements for let-7 function in the developing zebrashift embryo. Nucleic Acids Res 2004; 32: 6284–6291.
129. Abdelmohsen K, Srikantan S, Kuwano Y, Gorospe M. Mir-519 reduces cell proliferation by interfering with dicer function in the developing zebrafish embryo. Mol Cell 2010; 39: 8163–8172.
130. Surdzel I, Cabanski M, Dallmann I, Lyszczewicz M, Kraeger A, Ganser A et al. Enforced expression of mir-125b affects myelopoiesis by targeting multiple signaling pathways. Blood 2011; 117: 4328–4340.
131. Adilakshmi T, Sudo I, Tapinos N. Combinatorial action of mirnas regulates transcriptional and post-transcriptional gene silencing following in vivo pns injury. PLoS One 2012; 7: e33674.