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Elena Mogilyansky
Thomas Jefferson University

Isidore Rigoutsos
Thomas Jefferson University

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Review

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

E Mogilyansky[1] and I Rigoutsos*,[1]

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 pivotal 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?

[1]Computational Medicine Center, Thomas Jefferson University, Philadelphia, PA 19107, USA
*Corresponding author: I Rigoutsos, Computational Medicine Center, Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA 19107, USA. Tel: +1 215 503-6152, Fax: +1 215 503-0466; E-mail: isidore.rigoutsos@jefferson.edu
Keywords: cancer; 'expanded' model of miRNA targeting; microRNA; miRNA; miR-17/92 cluster; oncomir
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; Atf4, 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; IFNγ, interferon-γ; Isl-1, insulin gene enhancer protein; HC, hepatocellular carcinoma; HSC, hematopoietic stem cells; MAPK 14, mitogen-activated protein kinase 14; M-CSF, macrophage-colony stimulating factor; *MIR17HG*, the miR-17/92 cluster host gene (non-protein coding); miRNAs, microRNAs; MITF, microphthalmia-associated transcription factor; MLL, mixed-lineage leukemia; MS, multiple sclerosis; MSCV, murine stem cell virus; ncRNAs, non-coding RNAs; Nrf2, 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.[1,2] 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*.[3,4] In 2000, a second miRNA, the well-conserved let-7, was discovered and functionally characterized as important for *C. elegans* development.[5] Since then, thousands of miRNAs have been predicted and identified in animals, plants and viruses (see http://www.mirbase.org).[6–8]

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.[9] Analysis of 70 human B-cell lymphoma cases showed amplification of this region.[9] 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[10,11] 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.[12] 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'.[13]

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.[14,15] 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.[16,17] N-MYC also transcriptionally activates *MIR17HG*[18] as well as

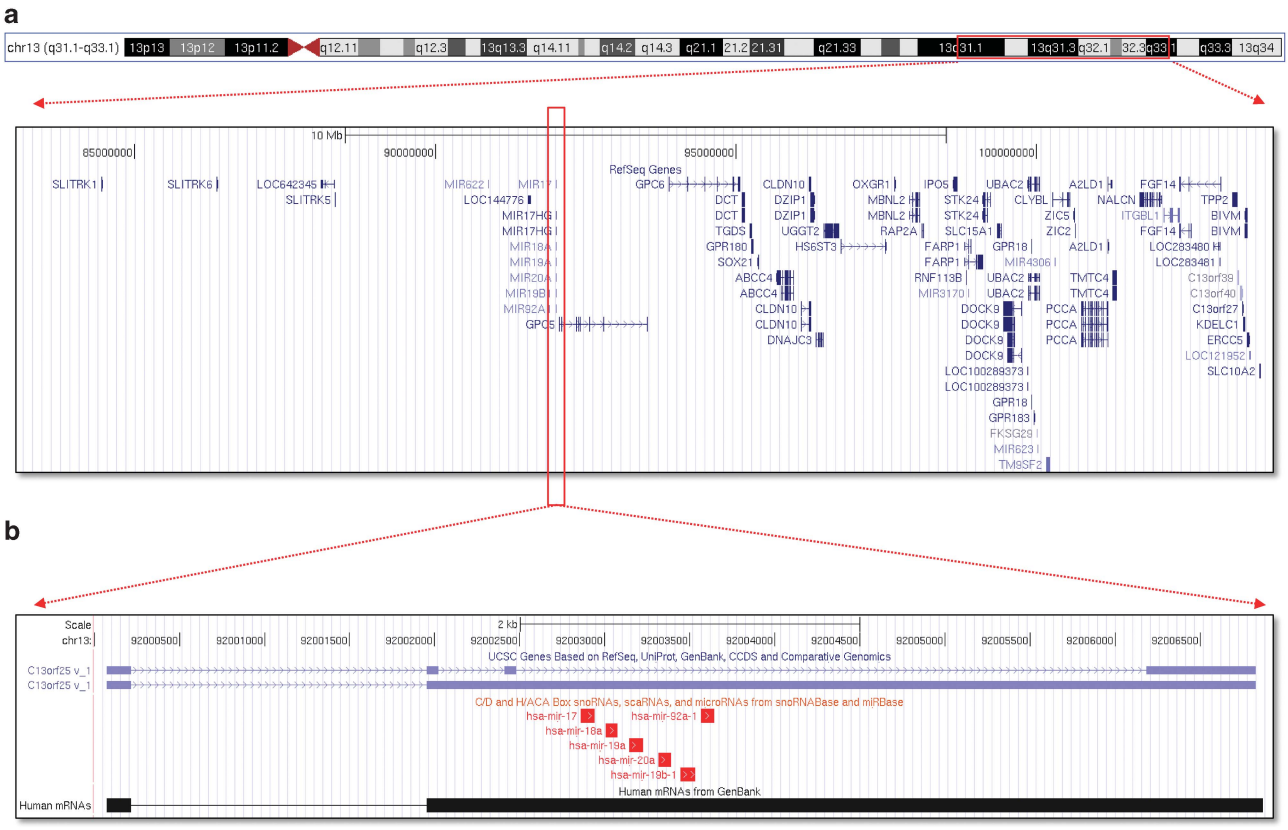

Figure 1 Genomic representation of the human miR-17-92 cluster host gene (*MIR17HG*) and neighborhood genes on Chr 13q31.1-q33-1. (**a**) Genomic representation of genes located ± 10 kb around human *MIR17HG*. (**b**) Genomic representation of *MIR17HG*. Two transcripts are shown in light blue and individual members of the cluster represented as red rectangles. The two panels were created using the UCSC genome browser (http://genome.ucsc.edu/)

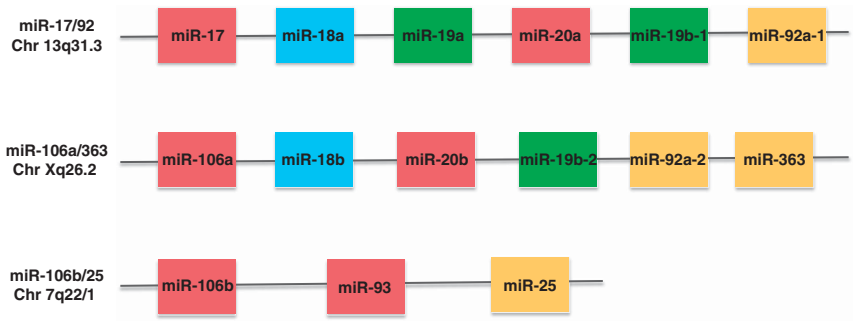

Figure 2 Members of the miR-17/92 cluster and its two paralogues miR-106a/363 and miR-106b/25 and their chromosomal location. Red: members of the miR-17 family; blue: members of the miR-18 family; green: members of the miR-19 family; orange: members of the miR-92 family

miR-17 family	**hsa-miR-17-5p**	C**AAAGUG**CUUACAGUGCAGGUAGU
	hsa-miR-20a-5p	U**AAAGUG**CUUAUAGUGCAGGUAG
	hsa-miR-20b-5p	C**AAAGUG**CUCAUAGUGCAGGUA
	hsa-miR-106a-5p	C**AAAGUG**CUAACAGUGCAGGUA
	hsa-miR-106b-5p	U**AAAGUG**CUGACAGUGCAGAU
	hsa-miR-93-5p	C**AAAGUG**CUGUUCGUGCAGGUAG
miR-18 family	**hsa-miR-18a-5p**	U**AAGGUG**CAUCUAGUGCAGAUA
	hsa-miR-18b-5p	U**AAGGUG**CAUCUAGUGCAGUUA
miR-19 family	**hsa-miR-19a-3p**	U**GUGCAA**AUCUAUGCAAAACUGA
	hsa-miR-19b-3p	U**GUGCAA**AUCCAUGCAAAACUGA
	hsa-miR-19b-3p	U**GUGCAA**AUCCAUGCAAAACUGA
miR-92 family	**hsa-miR-92a-3p**	U**AUUGCA**CUUGUCCCGGCCUG
	hsa-miR-92a-3p	U**AUUGCA**CUUGUCCCGGCCUG
	hsa-miR-25-3p	C**AUUGCA**CUUGUCUCGGUCUGA
	hsa-miR-363-3p	A**AUUGCA**CGGUAUCCAUCUGUAA

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

E2F1 and E2F3.[19,20] 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.[17,21,22] Indeed, some patients with an N-MYC mutation have developmental abnormalities similar to those in Feingold syndrome patients (see below).[23,24]

Additional information on transcriptional regulation became available through the recent release by the ENCODE (Encyclopedia of DNA Elements) project[25] of data from the study of 118 transcription factors (TFs). ENCODE's results revealed 1292 TF:miRNA interactions and 421 miRNA:TF interactions that in turn suggest tightly coupled auto-regulatory loops involving miRNAs and TFs. Of the 118 TFs, 34 pertain to the miR-17/92 cluster: they include the previously known MYC[17,18] and MXI[26,27] and 32 novel TFs (Figure 4). Among the newly identified TFs, BCL3 was found to regulate miR-17/92 and miR-106b/25. Further, *BCL3, IRF1, SP1, TAL1* and *ZBTB33* are targeted by individual miRNAs of the cluster, in addition to being TFs for the cluster (Figure 4). Moreover, several novel targets for members of miR-17/92 and miR-106b/25 were identified and are also summarized in Figure 4.[25,28] With regard to the miR-106a/363 cluster, it is likely regulated by the microphthalmia-associated transcription factor (MITF) through a binding site at position 133,135,780 (hg19) of chromosome X in the cluster's immediate vicinity.[29]

Among TFs, the E2F family (E2F1, E2F2 and E2F3) have a central role in the regulation of G1 to S phase progression.[30] All E2Fs,[17,19] especially E2F3,[20] have been shown to occupy miR-17/92's promoter region. E2Fs are also known to be targeted by miR-17/92, forming an auto-regulatory loop (Figure 4).[19,20] Finally, recent studies indicate that TP53 targets the miR-17/92 cluster[31] while also being targeted by miR-25 through regulation of the latter by Myc and *E2F1*.[32]

Despite these significant advances, knowledge about the transcriptional control of miR-17/92 and its paralogues remains largely fragmentary. It is also important to note that the specific processing of individual miRNAs adds a new level of complexity; that is, it is conceivable that there is a cell-type-dependent and context-dependent dimension to post-transcriptional silencing.[14,15,33,34]

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 *(TGFBRII)*, 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[15] and also by miR-106b/25.[39] During the endoplasmic reticulum related stress, unfolded protein response TFs, activating TFs, activating transcription factor 4 (Atf4) 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

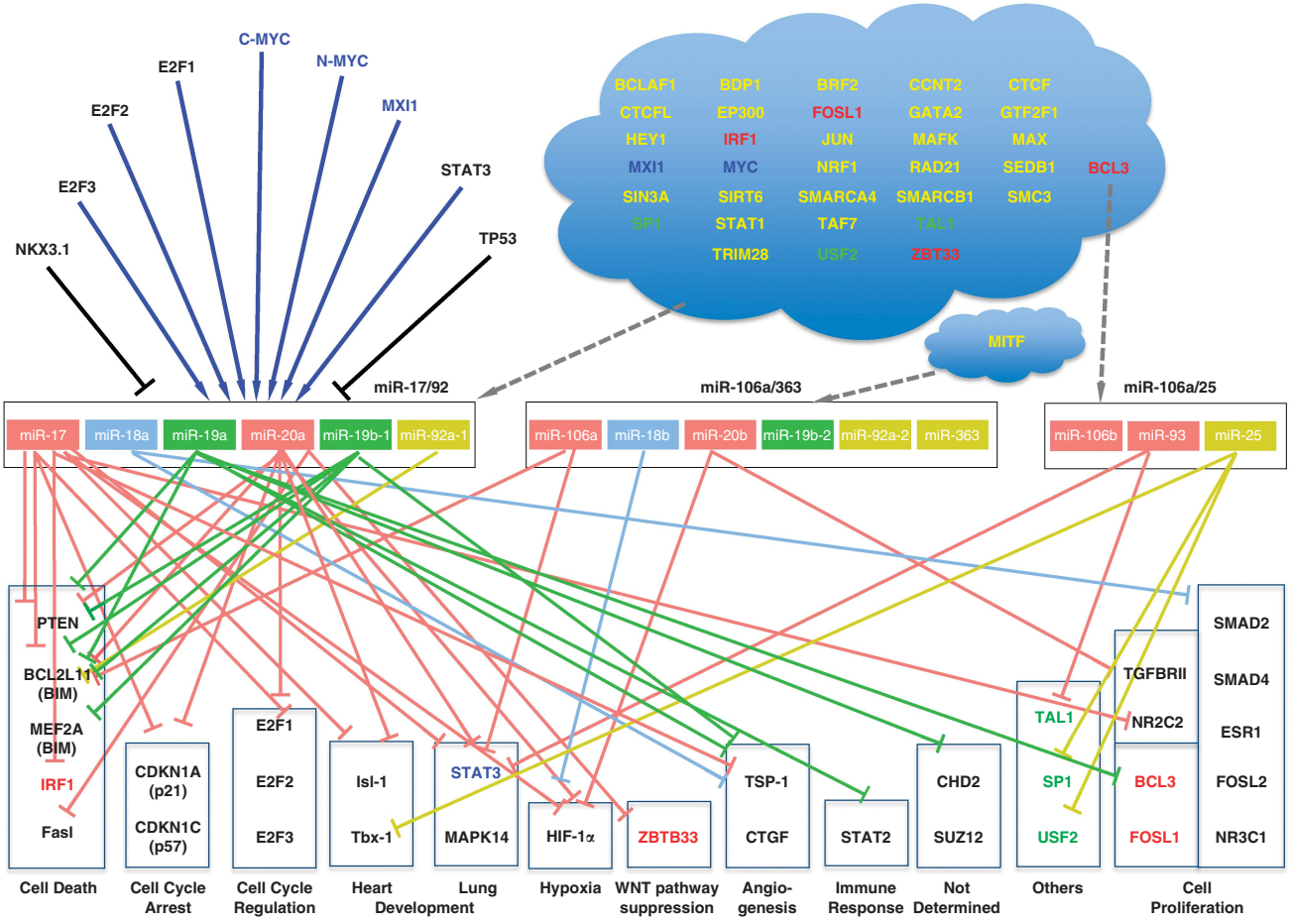

Figure 4 The transcriptional regulation and main targets of the miR-17/92 cluster and its paralogues. The transcriptional factors (TFs) in the left upper corner have been functionally validated; dark blue arrows indicate upregulation; black lines indicate repression. TFs in the blue 'cloud' were identified by the ENCODE project and the relationship of most of them to the miR-17/92 cluster and its paralogues is putative. Blue TFs were validated previously and confirmed by ENCODE; red TFs putatively regulate the miR-17/92 cluster and at the same time are known to be targeted by cluster members; green TFs putatively regulate the miR-17/92 cluster and at the same time are known to be targeted by paralogue miR-106b/25. If the specific gene that is targeted by a miRNA is known, the repressor line ends at the gene; otherwise, it ends at the box boundary of the respective cell process

tissue growth factor (*CTGF*).[40] 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).[41]

MiR-17/92 and Normal Development

The miR-17/92 cluster is highly expressed in embryonic cells[15] 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.[24] 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.[24]

Subsequent mouse studies showed that deletion of the miR-17/92 cluster is perinatal lethal.[15] 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.[15,22] 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*).[42] The overexpression of the miR-17/92 cluster leads to lung epithelium hyper-proliferation and suggests a role in lung cancer.[43]

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.[15] In experiments with adult mice whose hematopoietic system is reconstituted with fetal liver cells from a miR-17/92$^{\Delta neo/\Delta neo}$ 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.[15] Mice that were irradiated and had their hematopoietic system reconstituted with hematopoietic

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.[13] 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.[44]

Parallel studies have also implicated miR-17/92 in normal lymphocyte development.[45] 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 (IFNγ) production by miR-19b-1 and the promotion of a Th1 response by miR-17 and miR-19b-1.[45] Indeed, miR-17 and miR-19b-1 have an important role in promoting B-cell proliferation, protecting B-cells from death, supporting IFNγ production and suppressing T-cell differentiation.[45]

The Cluster as an Oncogene

We next review the increasing body of literature on the cluster's oncogenic role (Table 1).[46,47]

B-cell Lymphomas

The miR-17/92 cluster was initially found amplified in diffuse cell lymphomas.[9] Later, in B-cell lymphoma, an ectopically overexpressed truncated version that lacked miR-92 showed the cluster's role as an oncogene.[13] Moreover, as already discussed, c-Myc was shown to transcribe the truncated cluster in mouse models of B-cell lymphoma.[13] These findings represent early evidence that miR-17/92 can act as an oncogene by suppressing apoptosis.[13] MiR-18a levels in diffuse large B-cell lymphoma correlate strongly and negatively with survival (higher expression-shorter survival).[48] As mentioned already, miR-19a and miR-19b-1 are necessary and sufficient to promote tumorigenesis B-cell lymphoma.[49–51] 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.[49]

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.[52] 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-

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/Feingold syndrome	Lung and heart development	B-cell development	T-cell development	B-cell lymphoma	Diffuse large B-cell lymphoma	B-cell CLL	MLL-rearrangements	AML t (8;21)	AML t (15;17)	T-cell lymphoma	T-cell ALL	Multiple myeloma	Retinoblastoma	Colorectal cancer	Head and neck cancer	Pancreatic cancer	Breast cancer	Ovarian cancer	Lung cancer	Renal cancer	Hepatocellular carcinoma	Osteosarcoma	Gastric cancer	Nasopharyngeal cancer	Urothelial cancer	Autoimmunity	Cardiovascular diseases	Alzheimer's disease	Multiple sclerosis	Aging
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.

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 $\sim$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 $p21^{Cip1}$ and $p57^{Kip2}$.[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.[60] 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 meduloblastomas, 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 cancer[73] 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 homeotic 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-17/92 and its paralogues (miR-92,[83] miR-18a, miR-106b, miR-93 and miR-25[84]) 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 antagomiR-92a led to inhibition of miR-92a with consequent growth of new blood vessels and recovery from ischemia.[92] 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 $\sim$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 downregulation of miR-17, miR-19b, 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 Potentially Wide Spectrum of Coding and Non-coding Sequences Targeted by the Cluster

It should be clear by now that the cluster's members are involved in very diverse settings and a wide range of post-transcriptional regulatory events. The experimental findings to date about the cluster's targetome have been driven primarily by the analyses of gene-expression array data. The exact mechanistic aspects of those interactions have been elucidated in only relatively few contexts. To better appreciate the range of possibilities and to place the cluster's known (and unknown) targets in the appropriate light, it is important to first review briefly how miRNA target prediction has been practiced.

A miRNA effects the post-transcriptional regulation of its targets in a sequence-dependent manner. The 'seed' in particular, that is, the sequence spanning positions 2–7 inclusive from the 5′ end of a miRNA, is especially important for target determination and coupling.[1,5,107–113] Early genetic studies and subsequent efforts demonstrated that miRNAs can function in the presence of inexact matches and/or bulges in the seed region.[108,109,111,114–125] Despite the potential diversity of targets, there has been a strong adherence throughout to a 'standard' model that anticipates exact base-pairing, that is, Watson–Crick, and the absence of any bulges in the seed region. Consequently, the majority of the designed algorithms treated these criteria as necessary and sufficient attributes of *bona fide* targets and combined them with the enforcement of cross-genome conservation. However, algorithms were also proposed that neither enforced nor used these constraints, for example, rna22.[126] As a matter of fact, rna22 permits Watson–Crick and G:U pairs in the seed region as well as bulges; moreover, a candidate target does not have to be conserved across organisms and can be located anywhere.

An additional element, likely guided by the early *C. elegans* studies with the 3′UTR of the *lin-14* and *lin-41* genes, relates to an adherence to seek miRNA targets primarily in the 3′UTR of the candidate mRNAs. Nevertheless, the use of artificial constructs demonstrated the absence of any mechanistic constraints that would prevent the targeting of other mRNA regions by miRNAs: let-7 target sites embedded in the coding sequence (CDS) were shown to be functional.[127] Analogously, the introduction of miRNA target sites into the 5′UTR of luciferase reporter mRNAs containing internal ribosome entry sites (IRESs) revealed that miRNAs could regulate the abundance of a target by binding to its 5′UTR.[128]

Further support for these findings came more recently through a number of efforts by our group and others that reported the discovery and validation of extensive and naturally occurring miRNA targets in the CDS of an mRNA as well as its 5′UTR.[129–134] In earlier work, we estimated that as many as 90% of the known protein-coding human genes could be targeted by miRNAs.[126] On a related note, miRNAs were recently shown to also target intergenic and intronic regions, pseudogenes and long ncRNAs.[119,121,123,135,136]

Another dimension of 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.[137] However, not every known miRNA is conserved: indeed, there are reports of miRNAs that are species- or genus-specific.[138,139] 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[5] and is embryonic lethal.[140] In the fruit fly, let-7-knockout flies are externally normal but exhibit behavioral defects and juvenile features in their neuromusculature.[141]

In recent work[113] 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[119] and sequenced miRNA targets.[113] 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[109,111,116–118] as well as discussed in the literature.[110,112,124,130,142]

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.[143,144] 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,[136] 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.[119,123,145–147] Initial analyses of the available data have provided additional evidence that miRNA-targeted transcripts include numerous transcripts that are not protein-coding.[113]

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

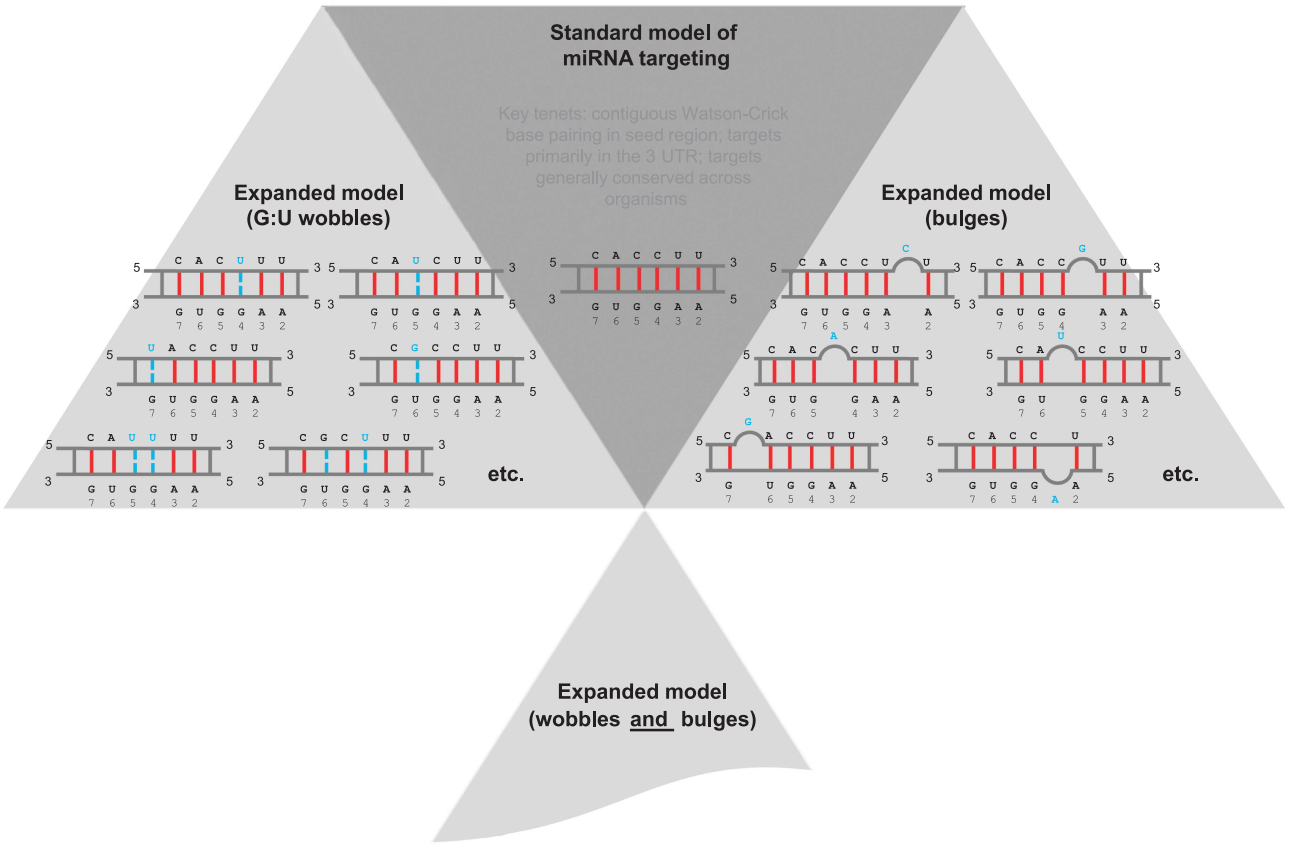

Figure 5 Differences between the 'standard' and 'expanded' model of miRNA targeting and the corresponding targetome. The seed of miR-18 is used as an example (bases at positions 2 through 7 inclusive). For the expanded model's G:U wobble examples and bulge examples, only a few representative cases are shown

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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