

Published in final edited form as:

Int J Biochem Cell Biol. 2010 August ; 42(8): 1348–1354. doi:10.1016/j.biocel.2010.03.004.

mir-17-92, a cluster of miRNAs in the midst of the cancer network

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Abstract

MicroRNAs (miRNAs) are an abundant class of small non-coding RNAs (ncRNAs) that function to regulate gene expression at the post-transcriptional level. Although their functions were originally described during normal development, miRNAs have emerged as integral components of the oncogenic and tumor suppressor network, regulating nearly all cellular processes altered during tumor formation. In particular, *mir-17-92*, a miRNA polycistron also known as *oncomir-1*, is among the most potent oncogenic miRNAs. Genomic amplification and elevated expression of *mir-17-92* were both found in several human B-cell lymphomas, and its enforced expression exhibits strong tumorigenic activity in multiple mouse tumor models. *mir-17-92* carries out pleiotropic functions during both normal development and malignant transformation, as it acts to promote proliferation, inhibit differentiation, increase angiogenesis and sustain cell survival. Unlike most protein coding genes, *mir-17-92* is a polycistronic miRNA cluster that contains multiple miRNA components, each of which has a potential to regulate hundreds of target mRNAs. This unique gene structure of *mir-17-92* may underlie the molecular basis for its pleiotropic functions in a cell type and context dependent manner. Here we review the recent literature on the functional studies of *mir-17-92*, and highlight its potential impacts on the oncogene network. These findings on *mir-17-92* indicate that miRNAs, together with protein coding genes, are integrated components of the molecular pathways that regulate tumor development and tumor maintenance.

Keywords

miRNAs; cancer; *mir-17-92*; *oncomir-1*

1-Introduction

1-1 miRNAs in post-transcriptional silencing

Data from comparative genomic studies have long implicated the functional importance of the non-protein coding regions of the genome, whose proportion increases as a function of genomic complexity (Mattick and Makunin, 2006). Extensive transcriptions have been observed from many of these regions, giving rise to numerous non-coding RNAs (ncRNAs) that regulate diverse developmental and physiological processes (Kapranov et al., 2007). We are only beginning to understand the realm of ncRNA functions, yet preliminary studies so far have revealed unexpected complexity and richness of their expression patterns, genomic structures and biological activities. In recent years, microRNAs (miRNAs) have emerged as a class of novel, small ncRNAs with potent capacity for gene regulation at the post-transcriptional level (Zamore and Haley, 2005, He and Hannon, 2004, Bartel, 2009, Ambros,

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2004). First identified as novel ncRNAs essential for larval developmental timing in *C. elegans* (Wightman et al., 1993, Lee et al., 1993), thousands of such small RNAs have been and are still being discovered in almost all organisms, ranging from viruses and single-celled green algae *Chlamydomonas* (Zhao et al., 2007, Molnar et al., 2007) to complex mammalian species (Lee and Ambros, 2001, Lau et al., 2001, Lagos-Quintana et al., 2001). Upon maturation, miRNAs recognize specific mRNA targets through imperfect sequence complementarity, and largely dampen their expression at the post-transcriptional level. Given the ability of miRNAs to downregulate many mRNA targets, this level of gene regulation may provide robustness in a diverse range of biological processes, due to the simultaneous regulation of many components of the signaling network.

Genes encoding miRNAs are often transcribed into precursor transcripts (pri-miRNAs) that contain a stem loop hairpin structure(s) (Lee et al., 2002). Pri-miRNAs are sequentially processed by two RNaseIII enzymes, Drosha and Dicer, to yield mature miRNA duplexes ranging from 18 to 24 nucleotide in length (Lee et al., 2003, Hutvagner and Zamore, 2002). One strand of the mature duplex is then incorporated into the effector complex, RISC, to mediate the post-transcriptional silencing of specific mRNAs (Hutvagner and Zamore, 2002, Mourelatos et al., 2002). The recognition of the miRNA targets often involves imperfect base pairing (Wightman et al., 1993, Lee et al., 1993), which allows miRNA to potentially regulate a large number of protein coding genes (Lewis et al., 2003, Lewis et al., 2005, Krek et al., 2005, Grimson et al., 2007). Interestingly, multiple miRNAs can be produced within a single pri-miRNA transcript, each of which can act independently. This polycistronic structure of miRNA cluster genes sets them apart from most protein coding genes, and bestows them with a unique capacity and specificity for gene regulation in the complex molecular network for development and disease.

Despite the small size of the miRNA molecules, they exhibit enormous capacity for gene regulation, mostly due to the nature of imperfect base pairing required for target recognition (Lewis et al., 2003, Lewis et al., 2005, Krek et al., 2005, Grimson et al., 2007). Therefore, each miRNA is theoretically capable of regulating hundreds of mRNAs within a cell type and in a context dependent manner. Initially, a small number of miRNA targets were identified based on classic invertebrate genetics studies (Johnston and Hobert, 2003, Brennecke et al., 2003, Wightman et al., 1993, Lee et al., 1993). Examination of these validated target sites combined with biochemical studies revealed that the 5' end of a miRNA, designated as the "seed" sequence, plays a critical role in target recognition and post-transcriptional repression (Lewis et al., 2005, Grimson et al., 2007, Doench and Sharp, 2004). The miRNA seed complementarity was employed to develop computational approaches for target prediction, and was later demonstrated experimentally as the major molecular basis for miRNA target recognition in cell culture systems (Selbach et al., 2008, Baek et al., 2008). Exceptions to this generalization still exist (Didiano and Hobert, 2006, Wightman et al., 1993). For example, in *C. Elegans*, a 6- to 8-base-pair perfect seed pairing is not always a reliable predictor for the *lisy-6* miRNA interaction with its targets, because it has been shown that G.U base pairing is tolerated in the 'seed' region. It is likely that a variety of mechanisms may act together to regulate the specificity of miRNA:target interaction (Didiano and Hobert, 2006).

The exact molecular basis underlying miRNA mediated gene silencing is not entirely clear. Biochemical studies using different reporter systems and endogenous miRNAs have yielded several distinct models, possibly reflecting the limitation of the *in vitro* reporter systems tested and/or the complex nature of miRNA mediated gene silencing (Liu et al., 2005, Kiriakidou et al., 2007, Eulalio et al., 2008, Chendrimada et al., 2007, Bhattacharyya et al., 2006). In all these models, miRNAs, upon maturation, are incorporated into the RISC (Hutvagner and Zamore, 2002), which mediates degradation of specific mRNAs (Eulalio et

al., 2008, Giraldez et al., 2006) and/or represses their translation (Bagga et al., 2005, Chendrimada et al., 2007, Kiriakidou et al., 2007, Pillai et al., 2005). The key components of the RISC are the Argonaute (Ago) proteins, which, through their interactions with the P-body components, mediate the formation of P-bodies that contain miRNAs and their targets (Eulalio et al., 2008, Liu et al., 2005, Sen and Blau, 2005). Within the P-body, miRNA targets are sequestered from the translational machineries and are subjected to mRNA degradation, at least partially, through deadenylation (Giraldez et al., 2006). In several other studies, Ago proteins are suggested to mediate translational repression through various mechanisms (Filipowicz et al., 2008). Although there are debates about the exact mechanisms of the miRNA mediated repression, these findings implicated that both mRNA destabilization and translational repression contribute to miRNA mediated gene silencing. This is consistent with the recent findings using genome wide quantitative mass spectrometry to characterize the impact of miRNAs on the proteome in a cell culture system (Baek et al., 2008, Selbach et al., 2008). It is likely that multiple molecular pathways, either individually or collectively, mediate the biological effects of miRNAs in a context dependent manner.

1-2 miRNAs in cancer biology

miRNAs were initially studied as key regulators for normal development, as mutations, either in the founding members of the miRNA family or in the key components of the miRNA biogenesis pathway, give rise to pronounced developmental defects in nearly all model organisms. Even though all miRNAs share significant similarities in their gene structures, biogenesis and effector machineries, their expression patterns and biological functions vary tremendously. This unique mechanism of posttranscriptional gene silencing, along with transcription regulation, translation control and post-translation modification, are major gene regulatory mechanisms in diverse developmental and physiological processes.

Shortly after the miRNA identification in mammalian species, it was recognized that miRNAs may not solely regulate developmental processes. Initial studies indicated that genomic alterations and expression dysregulation of miRNAs are frequently associated with various human cancers (Calin et al., 2004a, Calin et al., 2004b, Iorio et al., 2005, Lu et al., 2005). These early findings prompted a number of subsequent studies to explore the functional connections between miRNAs and malignant transformation in a variety of cancer types. Two major approaches were undertaken. One approach aimed to identify candidate oncogenic and tumor suppressor miRNAs for the transformation of a specific cell type. This often involved performing expression studies to compare miRNA profiles between tumor and normal tissues. Under this rationale, *mir-17-92* has been identified as a potential oncogene, due to its genomic amplification and elevated expression found in multiple hematopoietic malignancies, including diffuse large B-cell lymphomas (DLBCLs), mantle cell lymphomas and Burkitt's lymphomas (Hayashita et al., 2005). Previous cancer genomic studies on DNA copy number alteration and insertional mutagenesis screens in mice also provide insights into candidate oncogenic and tumor suppressor miRNAs that were not fully recognized before. For example, the oncogenic potential for *mir-155* and *mir-106a-92* in lymphomagenesis were identified through this approach (Kluiver et al., 2005, Uren et al., 2008). The other rationale aims to explore the molecular crosstalk between miRNAs and well-characterized cancer pathways. From expression studies, miRNA functional screens, and computational analyses, a handful of candidate miRNAs have emerged as key components of the oncogenic and tumor suppressor network. For example, it is through extensive expression studies that *miR-34* miRNAs were first identified as p53 transcriptional targets to mediate its tumor suppressor effects (Raver-Shapira et al., 2007, He et al., 2007, Chang et al., 2007); *mir-372* and *mir-373* were identified as potential oncogenes for testicular tumorigenesis out of a functional screen using a miRNA over-expression

library (Voorhoeve et al., 2006); and finally bioinformatic prediction of let-7 miRNA targets lead us to explore its roles in post-transcriptional repression on key oncogenes Ras and HMGA2 (Johnson et al., 2005, Park et al., 2007, Mayr et al., 2007). Altogether, these lines of investigation lead to a surge of interest in the functional studies of miRNAs in the oncogenic and tumor suppressor network, a topic that has been largely unexplored until recently.

2 *mir-17-92*, a polycistronic miRNA oncogene

2-1 The unique gene structure of *mir-17-92*

One of the best-characterized oncogenic miRNAs is *mir-17-92*, a polycistronic miRNA cluster also designated as *oncomir-1* (He et al., 2005). The precursor transcript derived from the *mir-17-92* gene contains six tandem stem-loop hairpin structures that ultimately yield six mature miRNAs, *miR-17*, *miR-18a*, *miR-19a*, *miR-20a*, *miR-19b-1*, *miR-92-1* (Tanzer and Stadler, 2004). Recent high-throughput parallel sequencing efforts also discovered the rare species of miRNAs* within this cluster, which are the complementary miRNAs derived from the opposite arms of each *mir-17-92* pre-miRNA. The six miRNAs encoded by *mir-17-92* can be categorized into three separate miRNA families according to their seed sequences — the *miR-17* family (including *miR-17*, *miR-20* and *miR-18*), the *miR-19* family (*miR-19a* and *miR-19b*) and the *miR-92* family (Fig.1). It is worth noting that *miR-18* exhibits a significant sequence homology with *miR-17* and *miR-20*, despite one nucleotide difference within the “seed” regions. Ancient gene duplications have given rise to two *mir-17-92* cluster paralogs in mammals — *mir106a-363* and *mir106b-25*, each of which only contains homologous miRNAs to a subset of *mir-17-92* components (Fig.1). Interestingly, not only the sequence of each miRNA component is highly conserved across species among all three paralogs, the organization of these miRNAs within *mir-17-92* family also exhibits high level of conservation. The functional significance of this conservation is still unclear. It possibly reflects the evolutionary path through which gene duplications followed by subsequent loss of individual miRNA component(s) shaped the formation of this polycistronic miRNA family.

Human *mir-17-92* is located at 13q31.3, a region amplified in several hematopoietic malignancies and solid tumors, including Diffuse B-Cell Lymphomas (DLBCLs), follicular lymphomas, Burkitt’s lymphomas and lung carcinoma (Ota et al., 2004). The minimal 13q31 amplicon characterized contains the *mir-17-92* precursor, whose elevated expression invariably correlates with the presence of the 13q31 amplicon (Ota et al., 2004, He et al., 2005). Over-expression of *mir-17-92* has been observed in multiple tumor types (Volinia et al., 2006, Petrocca et al., 2008a, He et al., 2005), yet the amplitude and frequency of its over-expression vary depending on the specific tumor type. For example, the Golub groups have examined the miRNA expression profiles in 334 tumor samples across multiple major cancer types, and the *mir-17-92* is predominantly over-expressed in the hematopoietic malignancies (Lu et al., 2005). Consistent with these findings, the murine *mir-17-92* and *mir106a-363* loci are common insertion sites for insertional mutagenesis screen in retrovirally induced erythroleukemia and T-cell leukemia further indicating the oncogenic potentials of this miRNA family (Uren et al., 2008, Cui et al., 2007, Wang et al., 2006, Landais et al., 2007)

2-2 *mir-17-92* as a potential oncogene

One of the first functional evidence to support the oncogenic activity of *mir-17-92* came from an *in vivo* mouse B-cell lymphoma model, in which enforced expression of *mir17-19b*, a truncated *mir-17-92* lacking *miR-92*, collaborated with *c-myc* oncogene to accelerate B lymphomagenesis (He et al., 2005). In this model, *c-myc* is driven by the immunoglobulin

heavy chain enhancer ($E\mu$) as a transgene. Elevated *c-myc* expression in the B-cell lineage promotes the formation of B-cell lymphomas with a relatively long latency. When *mir17-19b* is over-expressed in this model, it represses *c-myc* induced apoptosis, shortens the latency for tumor onset, and gives rise to highly invasive B-cell lymphomas. Interestingly, the over-expression of *mir17-19b* not only promotes the oncogenesis of *c-myc* expressing B-cells, but also alters the cell fate of the transformed B-cells. The majority of B-cell lymphomas derived from the $E\mu$ -*myc* transgene are more mature B-cells. Yet B-cell lymphomas resulted from the collaboration between *mir17-19b* and *c-myc* are mostly of progenitor B-cell origin, implicating a possible role of *mir-17-92* in B-cell development. This hypothesis was later validated by mouse genetic studies (Ventura et al., 2008). Deficiency of *mir-17-92* indeed affects B-cell development, particularly in the pro-B to pre-B transition, due to enhanced apoptosis occurring in the pro-B cells during both fetal and adult B-cell development (Ventura et al., 2008).

Following this initial observation, the effects of *mir-17-92* over-expression have been examined in multiple animal models, human cancers, and cell culture systems, for its abilities to regulate a number of cellular processes that favor malignant transformation. These studies altogether revealed the pleiotropic functions of *mir-17-92*, during both normal development and malignant transformation, to promote proliferation, inhibit differentiation, augment angiogenesis and sustain cell survival. It is worth noting that the exact functional readouts of *mir-17-92* in a given biological system are largely dependent on the cell type as well as the developmental contexts. For example, *mir-17-92* represses *c-myc* induced apoptosis in progenitor B-cells (Tagawa and Seto, 2005, He et al., 2005), and similarly, it inhibits cell death during pro-B to pre-B cell transition in normal development (Ventura et al., 2008). In contrast, *mir-17-92* enhances the proliferation of normal lung epithelial progenitor cells and malignant lung cancer cells, while inhibiting their differentiation (Hayashita et al., 2005, Lu et al., 2005). Interestingly, *mir-17-92* not only exhibits cell autonomous effects, but also performs cell non-autonomous functions. *mir-17-92* in endothelial cells augments angiogenesis during normal development (Suarez et al., 2008), yet *mir-17-92* up-regulation in cancer cells also promotes angiogenesis during tumor growth in a xenograft model (Dews et al., 2006).

Deletion of *mir-17-92* results in multiple development defects, including lung hypoplasia and ventricular septal defect. In particular, both fetal and adult B-cell development is significantly impaired due to the enhanced pro-B apoptosis during pro-B to pre-B transition (Ventura et al., 2008). Other hematopoietic lineages, including erythrocytes, granulocytes, monocytes, and T-cells, seem to be unaffected by the deletion of *mir-17-92* in the initial phenotypical characterization of the knockout mice. Interestingly, when Dicer was deleted specifically in B-cells, which abolished the entire miRNA biogenesis in the B-cell compartment, the resulting phenotype resembled that of *mir-17-92* knockout mice to a certain degree. There was an accumulation of pro-B and a decrease of pre- and mature B cells (Koralov et al., 2008). Therefore, at least a portion of the Dicer phenotype could be attributed to the deficiency of *mir-17-92*. These observations are in line with findings from transgenic studies, where enforced expression of *mir-17-92* in the lymphoid lineages promotes their proliferation and dampens the activation-induced cell death (Xiao et al., 2008). These mice develop lymphoproliferative disease and autoimmunity, suggesting that the pathological amplification of *mir-17-92* can lead to an expansion of cells harboring this lesion, subsequently increasing the probability of oncogenic mutations that eventually lead to malignancy.

2-3 The pleiotropic functions of *mir-17-92*

mir-17-92 is widely expressed in many different cell types. Its diverse functionality may result from the different target mRNAs subjected to *mir-17-92* post-transcriptional silencing

in different cell types and/or different developmental or physiological contexts. Yet the underlying molecular basis for its pleiotropic effects is still unclear. It is likely that the multiple miRNA components located within the *mir-17-92* cluster may contribute to its functional complexity. Theoretically, the six mature miRNAs will act independently yet coordinately once the *mir-17-92* pri-miRNA is cleaved by Drosha. This unique structural feature of the *mir-17-92* cluster confers enormous potential and plasticity for gene regulation, as different target mRNAs may be targeted by a combination of *mir-17-92* components, varying in the degree of binding affinity. Thus, multiple miRNA components within *mir-17-92* can offer a wide dynamic range for miRNA-mRNA interactions, and consequently may result in diverse biological outputs in different cell types under different developmental and/or physiological contexts.

The exact biological activities of each *mir-17-92* component have not been functionally dissected. However, mouse knockout studies have implicated that these *mir-17-92* components may perform specific yet overlapping functions, arguing against the model where each miRNA component contributes equally to the overall biological functions of *mir-17-92*. Ventura et al. have knocked out the *mir-17-92* cluster and its paralogous clusters, *mir106a-363* and *mir106b-25*, both individually and in combination. Since these three miRNA clusters share extensive homology within a subset of their miRNA components, their different knockout phenotypes implicate the different functions of the *mir-17-92* components.

It is very likely that *mir-106a-363* is not expressed during embryonic development, so it is not surprising that deletion of *mir106a-363* does not yield significant defects in embryonic development (Ventura et al., 2008). It is surprising, however, that deletion of the widely expressed *mir106b-25* cluster alone or in combination with *mir-106a-363* does not result in obvious phenotype, but the deficiency of *mir-17-92* gives rise to defective embryonic development and postnatal death (Ventura et al., 2008). Double knockout mice with deficiency in both *mir-17-92* and *mir106b-25* yield quantitatively more severe phenotypes as well as a few novel defects, implicating the existence of a possible redundancy between *mir-17-92* and *mir-106b-25*. *mir-17-92* and *mir106b-25* share extensive sequence homology within a subset of miRNA components, including *miR-17/20/106b/93* and *miR-92/25*. However, *miR-18* and *miR-19* family miRNAs, while present in *mir-17-92*, are absent in *mir106b-25*. Despite the phenotypical differences between *mir-17-92* knockout and *mir106b-25* knockout, they exhibit similar embryonic expression patterns (Ventura et al., 2008). Thus, it is tempting to hypothesize that *mir-17-92* knockout defects revealed the unique biological functions for *miR-18* and *miR-19* family miRNAs. Surprisingly, the double knockout mice for *mir-17-92* and *mir-106b-25*, which presumably removed functional redundancy for *miR-17*, *miR-20* and *miR-92* during embryonic development, not only yield quantitatively different phenotypes with an increase in severity, but also give rise to a few qualitatively different defects. These findings raised an intriguing hypothesis that each of the miRNA components within *mir-17-92* may perform specific functions, although a certain degree of functional overlap and cooperativity may exist among the different miRNA components.

We recently reported the functional dissection of the individual miRNA components of *mir-17-92* by assaying their tumorigenic potential *in vivo*. Using the E μ -*myc* mouse B-cell lymphoma model, we identified *miR-19* as the key oncogenic component of *mir-17-92*. *miR-19* is both necessary and sufficient for promoting *c-myc* induced lymphomagenesis through repression of apoptosis (Olive et al., unpublished results). Therefore, *mir-17-92* functions as a polycistronic miRNA cluster, whose miRNA components regulate gene expression either individually or in combination in a coordinated rather than an additive manner. However, more complex mechanism of coordination may also exist. Shan et al.

indicate in a recent study that *miR-17* overexpression in mice decreases cell proliferation, adhesion and migration, raising a possibility that components of *mir-17-92* can both positively and negatively regulate the same cellular process to achieve homeostasis *in vivo* (Shan et al., 2009).

2-4 The transcription and biogenesis of *mir-17-92*

Given the functional specificity of different *mir-17-92* components, it is likely that this miRNA polycistronic cluster is subjected to sophisticated transcriptional regulation. Due to the difficulty of defining the promoter region of ncRNAs in general, we are only beginning to reveal the transcriptional regulation for *mir-17-92* through the combined approach of expression screens and bioinformatics approaches. O'Donnell et al. have documented that the transcription of *mir-17-92* is directly activated by the *c-myc* oncogene (Fig.2) (O'Donnell et al., 2005), and not surprisingly, *N-myc* has also been shown to transcriptionally activate *mir-17-92* (Schulte et al., 2008). These findings are consistent with the functions of *mir-17-92* in promoting proliferation in a variety of cell types, including cells of lymphoid, epithelial, and neural origin. In addition to *myc*, E2F1 and E2F3 directly activate the transcription of *mir-17-92*, and chromatin immunoprecipitation demonstrates that E2F3 is the major E2F family member that binds to the *mir-17-92* promoter region (Sylvestre et al., 2007, Woods et al., 2007). E2Fs are essential for the progression of the cell cycle, as they activate a large number of S phase genes, including thymidine kinase, DNA polymerase, and Cyclins E and A. As a result, cycling cells are likely to have elevated *mir-17-92* due to the periodic burst of E2F activity during S phase, while quiescent cells may have reduced *mir-17-92* levels. This is consistent with the high level of *mir-17-92* expression in transformed cell lines, but not in the primary cell culture or fully differentiated cell types, suggesting *mir-17-92* a key component of the complex regulatory signaling network for cell proliferation. Interestingly, recent studies also indicate *mir-17-92* as a novel target for p53 mediated gene repression, most apparent in cells under hypoxia. Given the strong effect of *mir-17-92* to promote cell survival, it is likely that repression of *mir-17-92* by p53 gives rise to apoptosis under hypoxia treatment (Yan et al., 2009).

mir-17-92 is not only regulated by a network of transcriptional machineries, its biogenesis is also subjected to intricate regulation through a largely unknown mechanism. We are only beginning to appreciate this novel regulation on the miRNA pathway, the best example of which is the repression of *let-7* biogenesis by LIN-28 at both Drosha and Dicer cleavage (Heo et al., 2008, Newman et al., 2008, Viswanathan et al., 2008). It is recently identified that VEGF mediated up-regulation of *mir-17-92* only elevates the expression level of three miRNA components of the entire cluster, *miR-17*, *miR-18* and *miR-20*, to participate in the control of angiogenic phenotypes, suggesting a selective *mir-17-92* biogenesis under given biological contexts (Suarez et al., 2008). Recent studies have revealed the RNA binding protein hnRNPA1 is specifically required for processing of *miR-18* establishing a potential connection between the signaling transduction and specific miRNA biogenesis (Guil and Caceres, 2007). The specific processing of individual miRNAs within a polycistronic miRNA cluster leads to a new level of complexity in the regulation of miRNA expression and miRNA function in different cell types and under different contexts. It is also plausible that post-transcriptional silencing mediated by each miRNA component within *mir-17-92* may also be regulated in a cell type and context dependent manner.

2-5 Target identification of *mir-17-92*

One of the least understood questions about *mir-17-92* is the identity of its targets. Theoretically, each miRNA component within *mir-17-92* has the potential to regulate hundreds of specific mRNAs, as miRNA target recognition largely depends on sequence complementarities at the 6 nucleotide seed regions (Lewis et al., 2003, Lewis et al., 2005).

The post-transcriptional repression mediated by a specific miRNA often leads to moderate down-regulation of a large number of target mRNAs, which has been experimentally validated in two independent over-expression studies (Selbach et al., 2008, Baek et al., 2008). It is clear that *mir-17-92* mediated gene silencing plays important roles *in vivo* given its knockout phenotypes. The current model in the field would predict that *mir-17-92* gives rise to a moderate down-regulation of a large number of mRNAs in each cell type, which collectively mediates its biological functions. Yet it is still unclear if the physiological level of *mir-17-92* indeed represses a large number of target mRNAs in a given cell type, and if *mir-17-92* always leads to a moderate level of down-regulation of the key functional target mRNAs. The answer to these questions may be dependent on specific cellular context, and would require the disruption of the binding between *mir-17-92* and its specific target mRNA(s) *in vivo*. Using morpholino oligos (Choi et al., 2007) and targeted deletions (Dorsett et al., 2008), several groups have started to examine the biological effects of a specific miRNA-mRNA interaction. Such an approach is likely to resolve the above questions and to dissect the contribution of each target mRNA to the biological functions of *mir-17-92*.

Using bioinformatic predictions, western analysis, and reporter assays, recent studies have identified a number of *mir-17-92* targets, each of which is proposed to contribute to a specific functional readout of *mir-17-92* (Fig.2). For example, the anti-apoptotic effects of *mir-17-92* can be at least partially explained by its repression of Bim and PTEN, whose dysregulation may contribute to the increased apoptosis during pro-B to pre-B transition in *mir-17-92* deficient animals and the aberrant cell survival in the lymphoid lineages of the *mir-17-92* transgenic animals (Fig.2) (Xiao et al., 2008, Ventura et al., 2008, Koralov et al., 2008). It is interesting that E2F1 is also a target of *miR-17* and *miR-20* and that E2F2 and E2F3 are targets of *miR-20* (O'Donnell et al., 2005). Since E2F directly activates transcription of *mir-17-92* (Woods et al., 2007), these data suggest an auto-regulatory feedback loop between E2F factors and this miRNA cluster. This feedback possibly acts to maintain a homeostasis that dampens the apoptotic potential of E2F1 following a surge of proliferative signals. The proliferation effects of *mir-17-92*, on the other hand, can be partially attributed to its ability to repress the cyclin dependent kinase inhibitor CDKN1A (p21), a negative regulator of the G1-S checkpoint cell cycle progression. This repression is mostly achieved by *miR-17* and the related *miR-20* (Petrocca et al., 2008b, Ivanovska et al., 2008). Consistently, over-expression or inhibition of the *miR-17* family members is sufficient to promote or delay the entry of cells into S phase, respectively (Ivanovska 2008). The angiogenic activity of *mir-17-92* in tumor cells reflects its cell non-autonomous function. This effect of *mir-17-92*, at least in part, seems to be mediated by the direct repression on thrombospondin-1 (Tsp1) and connective tissue growth factor (CTGF) (Dews et al., 2006) (Fig.2). Altogether, these findings indicate the cell type specificity of *mir-17-92* targets.

2-6 Remaining questions

Although a majority of the literature characterized the oncogenic potential for *mir-17-92* from different angles, loss of heterogeneity at the 13q31.3 locus that harbors human *mir-17-92* has been reported in a few tumor types (Zhang et al., 2007), including a small percentage of ovarian and breast cancers and melanomas. Enforced expression of *miR-17* in breast cancer cell lines induces a reduction in proliferation of the cancer cells (Hossain et al., 2006), possibly through its repression on AIB1 (amplified in breast cancer 1), a transcriptional co-activator of E2F1 and of the estrogen receptor. These puzzling observations may reflect the functional complexity of *mir-17-92* in a context dependent and cell type dependent manner, and more experimental investigations are needed to fully explore the functional complexity of *mir-17-92*.

3. Summary

Recent studies have revealed the unexpected abundance and complexity of ncRNAs transcribed in the mammalian genome, the functions of which are largely unexplored. The identification of ncRNA components in the cancer pathway reflects the necessity for complex gene regulation to achieve homeostasis and flexibility to maintain a normal developmental or physiological state. *mir-17-92* is among the first miRNAs recognized as key components of the molecular network that impact tumorigenesis and tumor maintenance. Yet we are only beginning to understand its profound impacts and to appreciate its unique structural and functional relationship. The functional studies of *mir-17-92* have served as a paradigm to investigate the interplay between protein coding genes and non-coding genes, and to understand the unique structural features of polycistronic miRNA clusters. In addition, miRNAs, and more broadly, ncRNAs, represent a novel class of molecules that offer great promise for developing new diagnostic markers and therapeutic treatments. Understanding the functions of *mir-17-92* may serve as an entry point to explore the clinical applications of small RNA molecules that function in the oncogene and tumor suppressor network.

Acknowledgments

We thank Dr. Pengcheng Bu and Ms. Mona Foth for critical reading of this manuscript and for helpful discussions. L.H. is a fellow of the Searle Foundation and is supported by the K99 award from the NCI.

List of abbreviations

miRNA	microRNA
ncRNAs	non-coding RNAs

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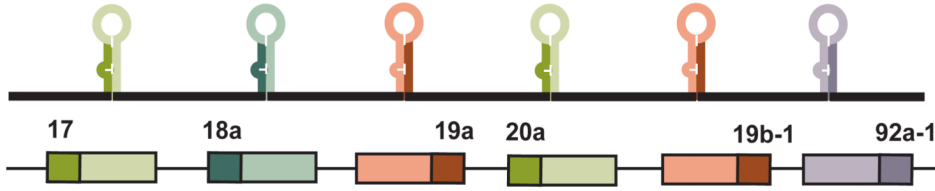
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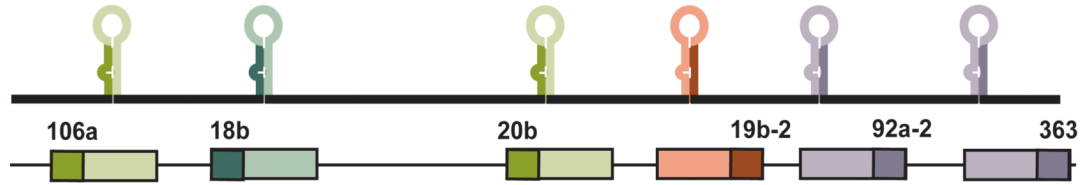
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Chr-13 *mir-17-92*



Chr-X *mir-106a-363*



Chr-7 *mir-106b-25*

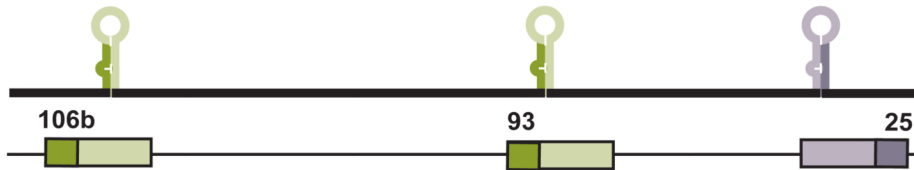


Fig1. Gene structure of human *mir-17-92* and its homologues

Primary transcript organization of the human *mir-17-92* and its paralogs, *mir106a-363* and *mir106b-25* clusters. The miRNAs encoded by the 3 clusters can be categorized into four separate miRNA families according to their seed sequences, including the *miR-17* family (*miR-17*, *miR-20a*, *miR-20b*, *miR-106a*, *miR-106b* and *miR-93*), the *miR-18* family (*miR-18a* and *miR-18b*), the *miR-19* family (*miR-19a*, *miR-19b-1* and *miR-19b-2*) and the *miR-92* family (*miR-92a-1*, *miR-92a-2*, *miR-383* and *miR-25*).

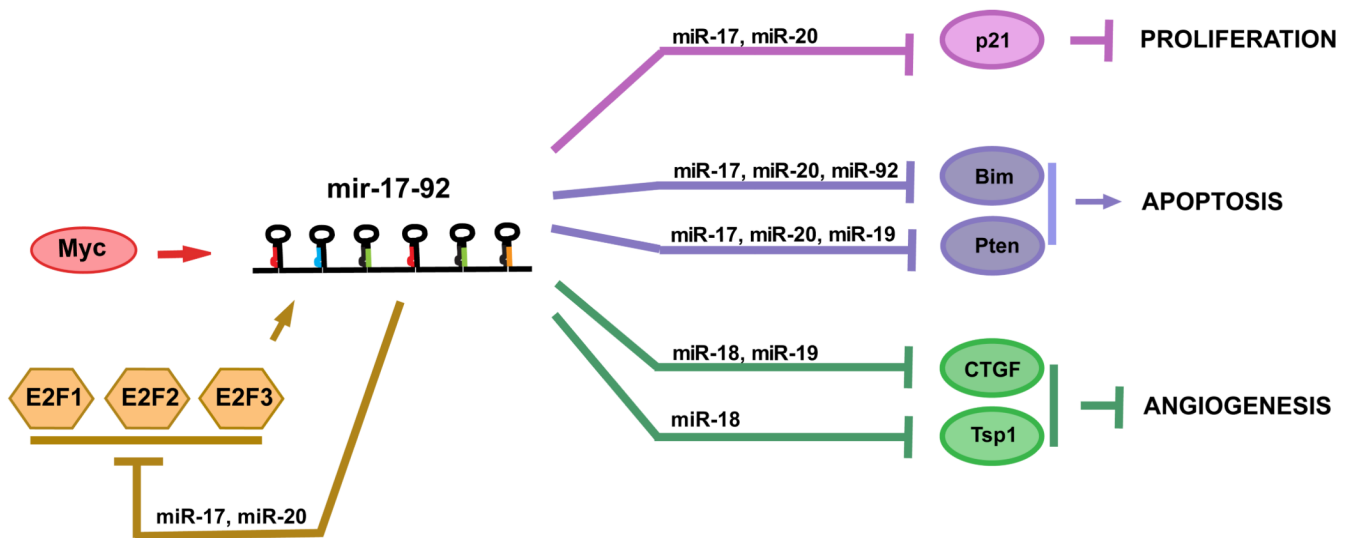


Fig2. The pleiotropic functions of *mir-17-92* achieved by repressing specific targets
Depending on both cell type and physiological context, *mir-17-92* can promote proliferation, increase angiogenesis and sustain cell survival through the post-transcriptional repression of a number of target mRNAs.