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***mir-17-92*, a polycistronic oncomir with pleiotropic functions**

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Abstract

Neoplastic transformation is caused by accumulation of genetic lesions that ultimately convert normal cells into tumor cells with uncontrolled proliferation and survival, unlimited replicative potential, and invasive growth. Emerging evidence has highlighted the functional importance of non-coding RNAs, particularly microRNAs (miRNAs), in the initiation and progression of tumor development. The *mir-17-92* miRNA is among the best characterized miRNA oncogenes, whose genomic amplification or aberrant elevation are frequently observed in a variety of tumor types. Unlike protein-coding oncogenes, where one transcript produces one protein, *mir-17-92* encodes a polycistronic miRNA transcript that yields six individual miRNA components. This unique gene structure, shared by many important miRNA oncogenes and tumor suppressors, underlies the unique functionality of *mir-17-92* in a cell type and context dependent manner. Recent functional dissection of *mir-17-92* indicates that individual *mir-17-92* components perform distinct biological functions, which collectively regulate multiple related cellular processes during development and disease. The structural complexity of *mir-17-92* as a polycistronic miRNA oncogene, along with the complex mode of interactions among its components, constitute the molecular basis for its unique functional complexity during normal and tumor development

The biogenesis and post-transcriptional gene silencing of microRNAs

microRNAs (miRNAs) encode a class of small, non-coding RNAs (ncRNAs) that regulate gene expression through post-transcriptional repression(1, 2). miRNAs can reside in introns and exons of protein-coding or non-coding RNA genes, or reside as independent loci(3). The majority of miRNA precursors, the pri-miRNAs, are transcribed by RNA polymerase II, and subsequently processed to yield mature miRNA duplexes ranging from 18-24 bp in length(3). Upon maturation, one strand of the miRNA duplex is selectively incorporated into the RNA-induced silencing complex (RISC), subsequently mediating the post-transcriptional gene silencing of specific mRNA targets through imperfect complementarity(4). The specificity of the miRNA-mRNA binding is often, although not exclusively, achieved by a perfect base-pairing at the miRNA “seed” region — the 2-8 nucleotides at the miRNA 5' end(5),(6). miRNA mediated post-transcriptional silencing can occur through degradation of target mRNAs(7, 8), and/or inhibition of protein synthesis at the initiation stage(9, 10). Due to their small size and imperfect base-pairing with the targets, miRNAs have the capacity to regulate many target mRNAs, therefore acting as global regulators for gene expression.

Pri-miRNAs contain either a single hairpin structure, or a tandem of hairpin structures. It turns out that 30% of miRNAs are transcribed as polycistronic miRNA clusters(11). The

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majority of the pri-miRNAs are regulated by the canonical biogenesis pathway, where each stem-loop hairpin structure was processed in a sequence-independent manner by the microprocessor complex that consists of a nuclear ribonuclease (RNase) III enzyme Drosha and a RNA binding protein Dgcr8(3). Dgcr8 binds to pri-miRNAs at the base of the hairpin stem structures and anchors the Drosha cleavage approximately 11 bp from this position(3). Subsequently, pre-miRNAs are transported into the cytoplasm by exportin-5 (Exp5), a Ran-GTP-dependent transporter, followed by a second cleavage mediated by another RNase-III enzyme Dicer to yield mature miRNA duplexes(3). For a small subset of the miRNA genes, two non-canonical pathways also regulate miRNA biogenesis, independent of Drosha or Dicer, respectively. Under one scenario, intronic miRNA precursors can be processed, via a splicing mechanism, into pre-miRNAs, with or without subsequent exocuclease trimming. Such pre-miRNAs are subsequently processed by Dicer to yield mature miRNAs. Under the other scenario, as represented by miR-451, the pre-miRNA generated by Drosha cleavage is cleaved by Ago2, yielding mature miRNAs with divergent 3' end possibly due to differential trimming by exonucleolytic digestion(12, 13). Despite the differences in the biogenesis pathways, all miRNAs function as potent regulators for post-transcriptional gene repression as described above.

Polycistronic structures of miRNA genes

Different from the classic mammalian protein-coding gene, where one transcript gives rise to one protein product, the polycistronic gene structure is prevalent among functionally important miRNAs. This unique gene structure allows the generation of multiple miRNAs from a single miRNA precursor, and draws a close analogy to polycistronic mRNAs commonly found in bacteria and archaea, where multiple functionally related protein coding genes are co-transcribed from a single promoter(14). Polycistronic miRNA genes come in different flavors, as some consist of a tandem of homologous miRNAs, some consist of a tandem of non-homologous components; yet still others contain both homologous and non-homologous miRNA components. Given the transcriptional coregulation of the polycistronic miRNA components, it is likely that this unique genomic organization facilitates co-regulation of functional related miRNA components. As described below, such unique gene structure of miRNA genes is likely to confer a complex mode of functional interactions among different polycistronic components, yielding unique gene regulatory capacity not commonly seen in mammalian protein coding genes.

For polycistronic miRNAs, multiple miRNA components are co-transcribed into a single precursor with a tandem of stem-loop structures. The majority of the miRNAs polycistrons are regulated by the canonical miRNA biogenesis pathway, where microprocessor complexes and/or Dicer could confer differential processing on individual components. Yet still a small subset of miRNA polycistrons is subjected to both the canonical and the non-canonical biogenesis. For example, in the mir141/451 miRNA cluster, miR-141 is sequentially processed by Drosha and Dicer through the canonical pathway, while miR-451 is processed non-canonically, first by Drosha, and subsequently by Ago2 cleavage and exonuclease trimming(12, 13). Under both scenarios, polycistronic miRNA components are processed individually, and are likely to yield different mature miRNA levels despite their co-transcription. In addition to potential differences in biogenesis, individual mature miRNAs encoded by a polycistron may have different turn-over rates, which further impact the relative abundance of the functional miRNA products. Taken together, the gene regulatory network imposed by a polycistronic miRNA could be much more versatile than previously appreciated, leading to a new level of functional diversity and complexity that have been largely unexplored.

The unique gene structures of a polycistronic miRNA cluster, *mir-17-92*

One of the best characterized polycistronic miRNAs is *mir-17-92*, a potent miRNA oncogene located at chromosome 13q31, a region amplified in Burkitt's lymphoma, diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, mantle cell lymphoma and lung cancer(15-18). Elevated *mir-17-92* expression has also been observed in a variety of hematopoietic malignancies(19), as well as multiple solid tumor types. The *mir-17-92* cluster produces a single polycistronic transcript that yields six individual mature miRNAs, including miR-17, 18a, 19a, 20, 19b, and 92a (Figure 1). Based on the sequence homology and seed conservation, the six *mir-17-92* components belong to four distinct miRNA families, *mirR-17* (including *miR-17* and 20), *miR-18*, *mirR-19* (including *miR-19a* and 19b), and *miR-92* family (Fig.1A). It is worth noting that although mature *mirR-18* shares extensive sequence homology with *mirR-17* and miR-20, its seed sequence differ by one nucleotide, which classify *miR-18* as a unique miRNA family by itself. The distinct mature miRNA sequence of each *mir-17-92* component determines the specificity of target regulation, and ultimately the specificity of functional readout (Fig. 1A). It is conceivable that the distinct biological effects of all four miRNA families will collectively contribute to the oncogenic activity of the *mir-17-92* polycistron. More interestingly, although all six *mir-17-92* components were transcribed as a single miRNA precursor, *miR-92* has a more ancient evolutionary history compared to the remaining *mir-17-92* components. *miR-92* is evolutionary conserved in vertebrates, chordates and invertebrates, while the remaining *mir-17-92* components are only found in vertebrate(20). Thus, the unique polycistronic structure of *mir-17-92* expands its gene regulatory capacity, as different miRNA components could confer specific yet overlapping effects in gene regulation. Interestingly, *mir-17-92* is well conserved in vertebrate species, not only at the level of mature miRNA sequences, but also in the genomic organization within the polycistron. In mammals, the two *mir-17-92* paralogues, *mir106a-363* and *mir106b-25*, encode only a subset of homologous *mir-17-92* components (Fig. 1B), whose genomic arrangement within the polycistron is conserved(21). The functional significance of this gene organization is still largely unclear, yet it may implicate the existence of conserved functional interactions among specific miRNA components, giving rise to unusual gene regulatory mechanisms during cancer development.

mir-17-92 in normal development and physiology

mir-17-92 promotes both cell proliferation and cell survival during normal development. Deletion of *mir-17-92* results in postnatal lethality with multiple developmental defects, including lung hypoplasia and ventricular septal defect(22). In addition, both fetal and adult B-cell development is significantly impaired due to the enhanced pro-B apoptosis during the pro- to pre-B transition(22). These observations are in line with findings from transgenic studies, where enforced expression of *mir-17-92* in the lymphoid lineages promotes cell proliferation, leading to lymphoproliferative disease and autoimmunity(23). Functional redundancy was readily detected among the three *mir-17-92* homologues, as double knockout (KO) of *mir-17-92* and *mir106b-25*, or the triple KO mice, exhibited embryonic lethality with more severe phenotypes. Compared to the *mir-17-92* KO mice, the DKO and TKO mice not only exhibited the same lung hypoplasia and ventricular septal defects to a greater severity, but also carry extensive apoptosis in additional organs, including the fetal liver and the spinal cord(22).

Consistent with the lung hypoplasia caused by *mir-17-92* deficiency, elevated *mir-17-92* expression is also observed in human lung alveolar multipotent cells (24). At least in the E-Cad/Lgr6⁺ putative lung stem cells, the p38a and *mir-17-92* constitute an intricate molecular network to maintain the proper balance between human lung stem cell (HLSC)

self-renewal and differentiation(24). *mir-17-92* directly targets C/EBP α and GATA6 to repress HLSC differentiation into lung epithelia, while p38 α activation could counter this effect by repressing *mir-17-92* expression through p53 dependent transcriptional repression(24). In a separate study, overexpression of *mir-17-92* in embryonic lung epithelia promotes differentiation of proximal epithelia, but causes excessive proliferation of distal epithelial progenitors(25).

In addition to its roles in normal development, *mir-17-92* also plays a role in regulating the physiological functions of many cell types. In the adaptive immune system, *mir-17-92* is indispensable for T lymphocytes' effector responses towards antigen(26). In CD4⁺ T cells, *mir-17-92* functions as a comprehensive regulator that controls the full spectrum of CD4⁺ T cell surveillance against solid tumor. The Pten suppression by *miR-19b*, supplemented by *miR-17*-mediated inhibition on TGF β RII and CREB1, *mir-17-92* enhances antigen stimulated T cell proliferation, suppresses activation induced T cell death, supports IFN- γ production from lineage-biased type 1 helper T cells, and inhibits the differentiation of inducible regulatory T cells, the immunosuppressive T-cell lineage facilitating immune escape of tumor cells(26). In CD8⁺ T cells, *mir-17-92* exhibits a dynamic expression regulation during the acute anti-viral responses, being strongly induced at the peak of effector responses, but greatly dampened during the formation of memory CD8⁺ T cells. Coordinately, this cluster is critical for effector CD8⁺ T cell expansion; and, with enhanced mTOR signaling, the forced continuous expression of *mir-17-92* greatly impaired the CD8⁺ T cell memory differentiation(27).

Functional dissecting the oncogenic role of the *mir-17-92* polycistron

The connection between miRNAs and cancer was first suggested by their frequent genomic alteration and dysregulated expression in various human tumors(28). Multiple miRNAs were subsequently identified to promote or suppress oncogenesis as integral components of the molecular network of cancer. *mir-17-92* is one of the most potent miRNA oncogenes, located at chromosome 13q31, a region amplified in Burkitt's lymphoma, diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, mantle cell lymphoma and lung cancer(21). Increased *mir-17-92* expression was observed in a large cohort of human B-cell lymphomas and multiple solid tumor types as well(19, 29). The oncogenic potential of *mir-17-92* was initially examined in the *E μ -myc* mouse model of Burkitt's lymphoma. The *E μ -myc* transgene places a *c-myc* protooncogene downstream of the immunoglobulin heavy chain enhancer *E μ* , driving aberrant *c-myc* overexpression specifically in the B-cell compartment to promote the development of late onset B-lymphoma(30). Enforced expression of *mir17-19b*, a truncated *mir-17-92* cluster with the *miR-92* deletion, significantly accelerated *c-myc* induced B-lymphomagenesis due to impairment of *c-myc* induced apoptosis(31). These findings constitute some of the first functional evidence demonstrating the role of *mir-17-92* as a potent miRNA oncogene.

mir-17-92 is highly enriched in many tumor types including many hematopoietic malignancies, medulloblastomas, lung cancer, colon cancers and liver cancers(21). Oncogenic cooperation with *mir-17-92* was observed for many classic oncogenic lesions both *in vivo* and *in vitro*. For example, overexpression of *mir-17-92* in *Ink4c*^{-/-}; *Ptch1*^{+/-} granule neuron progenitors gave rise to medulloblastomas in orthotopic transplants with complete penetrance(32). In a mouse model for MLL leukemia, the increased level of the miR-17-19b enhanced the frequency of leukemia stem cells, coupling a stringent differentiation block and with enhanced proliferation(33). Enforced expression of *mir-17-92* also collaborated with deficient *Rb* pathway to promote retinoblastomas(34). *mir-17-92* is an oncogene with pleiotropic functions in regulating multiple cellular processes that contribute

to tumor development. Its overexpression in tumor cells enhances cell proliferation, evades apoptotic response, triggers angiogenesis, blocks differentiation and promotes metastasis.

The polycistronic structure of *mir-17-92* raises the question about the contribution of individual *mir-17-92* components to the overall oncogenic activity under different biological contexts. In the *Eμ-myc* model of Burkitt's lymphoma, aberrant c-Myc activation in normal B-cells elicits strong proliferative effects coupled with a p53-dependent apoptotic response(35). The convergence of these opposing cellular processes upon aberrant c-Myc activation enables a self-defense mechanism against uncontrollable cell expansion. The enforced *mir17-19b* expression activates the PI3K/AKT pathway, therefore repressing c-Myc induced apoptosis to promote B-lymphoma development (31, 36). Further functional dissection of *mir-17-92* under this biological context indicates the functional divergence of *mirR-17* and *mirR-19* miRNAs. The *mirR-19* family miRNAs, when overexpressed individually, strongly repress c-Myc induced apoptosis in premalignant B-cells, thus acting as the key oncogenic *mir-17-92* components to promote B-lymphomagenesis (36). Consistent with this finding, *mirR-19* miRNAs are also important for B-lymphoma maintenance, as *mirR-19* miRNAs rescued the apoptosis phenotype caused by acute *mir-17-92* deletion in the *Eμ-myc* B-lymphoma cells (37). The survival effect of *mirR-19* is mostly mediated by the increased PI3K/AKT signaling, largely due to the post-transcriptional repression of Pten by *mirR-19* (36-38). On the other hand, the *mirR-17* family miRNAs did not contribute significantly to the oncogenic cooperation between *mir-17-92* and c-Myc (36, 37). It is demonstrated that *mirR-17* plays an essential role in promoting cell proliferation largely by repressing the cyclin-dependent kinase inhibitor, p21 (34, 39, 40). Yet in the context of c-Myc induced B-lymphomagenesis, c-Myc itself promotes excessive cell proliferation and transcriptionally repress p21(41), thus making *mirR-17* miRNAs functionally redundant during the malignant transformation. The function of *miR-92* in the *Eμ-myc* model has not been well characterized so far, yet in several solid tumors, such as colon cancers, miR-92a is differentially upregulated compared to the other *mir-17-92* components, with an important role in promoting cell proliferation and cell survival *in vitro*(42).

The oncogenic lesion that cooperates with *mir-17-92* determines the identify of its key oncogenic component(s). Although *mirR-17* miRNAs are dispensable for promoting transformation of *Eμ-myc* B-cells, their functions are essential for the oncogenic activity of *mir-17-92* under other contexts. The enforced *mir-17-92* expression cooperates with *Rb* and p107 loss to promote the formation of high-grade retinoblastomas with frequent brain metastasis(34). Consistent with this finding, *mir-17-92* is also essential for retinoblastoma maintenance, as deficiency of *mir-17-92* suppresses retinoblastoma formation in mice harboring *Rb*, *p107* and *p53* mutations(40). Unlike the *Eμ-myc* lymphoma models, the key oncogenic functions of *mir-17-92* in retinoblastomas are not mediated by the *miR-19/Pten* axis that promotes apoptotic evasion. Instead, the proliferative *mir-17-92* components, particularly *miR-17/20*, became the key oncogenic miRNAs under this context. Suppression of *miR-17/20*, but not *mirR-19* miRNAs, significantly reduced cell proliferation in *Rb* deficient human retinoblastoma cell lines(34), and the inhibition of proliferation is more prominent when *miR-17/20* was co-suppressed with *p53* and *Rb* deficient human retinoblastoma cells (40). The functional importance of *miR-17/20* under this biological context is largely due to its repression of the cyclin-dependent kinase inhibitor *p21*, whose compensatory upregulation in retinal cells is robustly induced by *Rb* and/or p107 deficiency as a mechanism to halt tumor progression(34). Under other contexts, the pro-proliferative function of *miR-17* family miRNAs is also attributed to their ability to downregulate Tgfb pathway components.

The diverse functional capacity of *mir-17-92* as an oncogene is likely attributable to the multiple miRNA components encoded by its polycistronic gene structure. It becomes increasingly clear that the functional significance of each *mir-17-92* miRNAs varies in a cell type- and context-dependent manner. When acute oncogene activation induces apoptosis as a barrier for tumor progression, the *mirR-19* miRNAs could provide strong selectively advantages, at least in part, by targeting negative regulators of the PI3K/Akt pathway, Pten and PP2A(Olive et al., 2009; Mu et al., 2009). Thus, *mirR-19* miRNAs constitute the key *mir-17-92* components for its oncogenic cooperation with c-Myc in Burkitt's lymphoma, and with activated Notch1 in T-cell acute lymphoblastic leukemia (T-ALL) (38). On the other hand, when the block of cell proliferation becomes the rate limiting step for tumor initiation or progression, it is conceivable for *mirR-17* miRNAs to emerge as the key functional component of *mir-17-92*. Hence, the repression of *p21* by *mirR-17* and 20 is both necessary and sufficient to confer resistance to Ras induced senescence in primary fibroblasts(39), and to promote oncogenic collaboration with *Rb* and p107 deficiency in retinal cells(34).

The gene structure of *mir-17-92* confers unique versatility to its oncogenic functions due to the combined effects of its miRNA components. It is conceivable that the *miR-17*, 19 and 92 family miRNAs, or a subset of these, function cooperatively to promote tumor development. In addition to cooperative effects, there exist other complex modes of interactions among *mir-17-92* components. For example, neonatal mice overexpressing *miR-92* ultimately developed occurrence of erythroleukemia by promoting erythrocyte differentiation and transformation(43). It was implicated that *miR-92* directly targets *Gata1* and *p53* to promote erythroleukemia formation. However, a strong oncogenic cooperation is observed between *miR-92* and p53 deficiency to promote erythroleukemia, indicating a *miR-92* dependent transformation mechanism can be independent of p53. Thus, p53 may not be the major *miR-92* target underlying its oncogenic function (43). Surprisingly, neonatal mice infected with a retrovirus overexpressing the entire *mir-17-92* polycistron do not develop erythroleukemia, instead they showed a significant increase of multipotent hematopoietic progenitor cells both in the bone marrow and in the spleen(43). The discrepancy of the *mir-17-92* and *miR-92* phenotype suggested an antagonistic interaction between *miR-92* and other *mir-17-92* components in promoting erythroleukemia. Consistent with this idea, *miR-92a*-induced erythroleukemia development was abrogated by coexpression of *miR-17*. In this specific biological context, *mirR-17* directly regulates survival proteins Bcl-2, STAT-5, and Jak2, which caused reduced proliferation and elevated apoptosis in *miR-92* overexpressing erythroleukemia cells(43). Similarly, overexpressing *miR-19*, but not *mir-17-92*, led to the overproliferation of B-cells that were primed for malignant transformation(43). This finding suggested an antagonistic interaction between *mirR-19* and other *mir-17-92* components, yet the nature of such interactions has not been characterized so far. Since all *mir-17-92* components are co-regulated transcriptionally, the differential expression of different miRNAs is likely a result of differential miRNA biogenesis and/or turnover.

The antagonistic interaction between *mirR-17* and *miR-92*, particularly, the anti-proliferation and pro-apoptotic role of *miR-17*, came as a surprise, since majority of the studies characterized *miR-17* as a pro-proliferative miRNA that contribute to oncogenesis in many cell types. is quite surprising, as the underlying mechanism contradicts with the well-characterized oncogenic effects of *mirR-17* in promoting cell proliferation. However, several studies supported a role of *mir-17-92*, particularly, *miR-17*, to suppress cell proliferation under specific contexts. For example, transgenic mice overexpressing *mirR-17* showed overall growth retardation, smaller organs and greatly reduced hematopoietic cell lineages, largely due to decreased cell adhesion, migration and proliferation(44). Consistently, enforced *mirR-17* expression also reduced proliferation in breast cancer cells, at least in part,

due to repression of the AIB1 gene(45). It is not clear how *mirR-17* could confer opposing effects on proliferation under different biological contexts, yet the likelihood of such is further supported by the occurrence of heterozygous deletion of *mir-17-92* in several solid tumor types, including ovarian cancers, breast cancers, and melanomas(46). Given the overwhelming evidence suggesting the role of *mir-17-92* as an oncogene, this paradoxical tumor suppressive role of *mir-17-92* remains largely unexplored. At least, these unexpected findings implicated the plasticity of *mir-17-92* functions under different biological contexts, which is likely dictated by the target specificity and the extent of posttranscriptional repression.

The biological effects of *mir-17-92*, and its components, are also heavily dependent on the cell types. For example, *mir-17-92* exhibited potent, yet opposing effects on angiogenesis in tumor cells versus endothelial cells. Aberrant pathological elevation of *mir-17-92* in tumors promotes angiogenesis in a non-cell autonomous manner, predominantly by Tsp1 and CTGF repression through *mirR-18* and miR-19(47). Surprisingly, physiological expression of *mir-17-92* represses endothelial cell sprouting through coordinated effects of all the miRNA components in a cell autonomous manner, with *mirR-17* yielding the strongest effects by targeting Jak1(48). This unexpected complexity, again, conferred by the multiplicity of the encoded components, highlighted the unique functionalities of polycistronic miRNAs.

Transcriptional regulation of *mir-17-92*

A number of oncogenic transcription factors were shown to regulate the expression of *mir-17-92* collaboratively. c-Myc is the first identified transcription regulator of *mir-17-92* in multiple cell types (49). Similarly, *mir-17-92* can also be upregulated by N-Myc in neuroblastoma cells to promote oncogenesis and repress the Tgfb pathway through downregulating multiple key Tgfb effectors(50). Like most Myc targets, the extent of *mir-17-92* upregulation by Myc is rather modest. In contrast, E2F family transcription factors, E2F1, 2 and 3, are potent inducer of *mir-17-92*, which acts as the key mediator for promoting cell proliferation downstream of E2F transcription factors(51). In Friend's leukemia, Spi-1 and Fli-1, two ETS family transcription factors that constitutes the key oncogenic lesions, directly activate the transcription of *mir-17-92* through the binding to a conserved ETS site. Induction of *mir-17-92* by Spi-1 and Fli-1 partially mediates the proliferative effects on the erythroleukemic cells(52). *mir-17-92* is also subjected to transcription repression by key tumor suppressors. *mir-17-92* cluster were transcriptionally repressed in hypoxia-treated cells through a p53-dependent pathway, which gave rise to apoptotic response (Yan et al, 2009).

In addition to transcription factors, histone modification provides another level of transcriptional regulation for *mir-17-92*, as methylation of histone 3 lysine 4 has been implicated in regulating *mir-17-92* expression. In MLL leukemias, several MLL fusions directly bind to the *mir-17-92* promoter, causing aberrant histone H3 acetylation and H3K4 trimethylation to upregulate *mir-17-92* expression(53). In another study, the transcription factor PU.1 indirectly represses *mir-17-92* expression to promote myeloid differentiation. This is largely achieved by PU.1 induction of Egr2, which recruits histone demethylase Jarid1b to the *mir-17-92* promoter to mediate histone H3 lysine K4 demethylation(54). In this case, a negative feedback was also observed between *mir-17-92* and Egr2 in a subset of acute myeloid leukemias (AML), where elevated *mir-17-92* downregulates Egr2 to reinforce its own upregulation(54).

Differential expression of *mir-17-92* components

Although transcriptional activation promotes the generation of pri-*mir-17-92*, post-transcriptional regulation of this polycistron has established differential expression of individual mature miRNAs (Fig.2). This is first demonstrated by O'Donnell et al, where they observed different levels of c-Myc induction for individual *mir-17-92* components, with the mature *mirR-18* being the most elevated and the mature *miR-92* the least(49). Subsequently, similar observations were made in many other biological systems, yet the relative abundance of individual *mir-17-92* components varies depending on the biological systems. For example, in germinal center diffuse large B-cell lymphomas (GC-DLBCLs), *miR-20a* is the most upregulated *mir-17-92* component(55). In multiple myelomas, *miR-17*, *20a* and *92* are more upregulated in patients with poorer prognosis(56). Given the unique functional readout of individual *mir-17-92* miRNAs, along with their complex interactions, the exact level of each *mir-17-92* component is likely to play an important role in determining its functional readout.

It is still unclear what determines the relative abundance of individual *mir-17-92* components. First, the tertiary structure of *mir-17-92* is clearly important for the pri-miRNA to pre-miRNA processing, as a mere shuffling of pre-miR hairpin structures is sufficient to alter their relative abundance(57). Efficient Drosha processing of pri-miRNAs requires the DGCR8 binding to the junction between single and double stranded RNA. The access to such recognition site could differ for each pre-miRs due to the impact of the tertiary *mir-17-92* structure(57). Second, it is possible that specific RNA binding protein(s) interacts with individual pre-miRNA component of *mir-17-92* to modulate its biogenesis (Fig. 2). This is best demonstrated in the case of miR-18, whose cleavage by Drosha is facilitated by its binding to hnRNP A1(58). Finally, the stability of the pre- and/or mature *mir-17-92* components could also be differentially regulated, leading to different turnover rate of individual miRNAs (Fig. 2). Among the best examples for such regulation is the polyuridination of *let-7* pre-miRNA. An RNA binding protein Lin28a interacts with *pre-let-7* to recruit the terminal uridin transferase TUT4 to mediate the polyuridination, and subsequently the degradation of *pre-let-7*(59). It is likely that similar mechanisms exist to regulate the turnover rate of different *mir-17-92* components in a cell type and context dependent manner.

Summary

We are only beginning to understand the realm of non-coding RNA functions during tumor development. *mir-17-92* encodes a polycistronic oncogenic miRNA essential for many developmental and pathogenic processes. Due to their small size and imperfect base-pairing with the mRNA targets, miRNAs generally have the capacity to regulate many target mRNAs, and therefore act as global regulators for gene expression. The polycistronic gene structure of *mir-17-92* allows co-transcription of six individual miRNA components. The enormous gene regulatory capacity of each *mir-17-92* miRNA, the complex coordination among different *mir-17-92* components, and their differential expression at the mature miRNA level, all constitute the molecular basis for the pleiotropic biological functions of this miRNA polycistron. These findings on *mir-17-92* provide a paradigm to explore the unique structures and functions of polycistronic miRNA oncogenes and tumor suppressors, and will ultimately reveal the unusual gene regulatory mechanisms unique to non-coding RNA genes.

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Fig. 1. *mir-17-92* encodes a polycistronic miRNA oncogene. **A.** The gene structure of the *mir-17-92* polycistron. *mir-17-92* encodes a miRNA precursor that yields 6 mature miRNAs. Based on seed sequence homology, these six miRNAs belong to four miRNA families. Homologous miRNA components are indicated by the same color. **B.** *mir-17-92* has two closely related homologues in mammals. The *mir-106a-393* and *mir-106b-25* clusters contain homologous miRNAs to a subset of *mir-17-92* components with a conserved genome arrangement.

Fig. 2.

A diagram of possible regulatory mechanisms that collectively modulate the biological functions of the *mir-17-92* polycistronic oncomir.