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miR-17∼92 in lymphocyte development and lymphomagenesis

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ABSTRACT

microRNAs (miRNAs) down-modulate the levels of proteins by sequence-specific binding to their respective target mRNAs, causing translational repression or mRNA degradation. The miR-17∼92 cluster encodes for six miRNAs whose target recognition specificities are determined by their distinct sequence. In mice, the four miRNA families generated from the miR-17∼92 cluster coordinate to allow for proper lymphocyte development and effective adaptive immune responses following infection or immunization. Lymphocyte development and homeostasis rely on tight regulation of PI3K signaling to avoid autoimmunity or immunodeficiency, and the miR-17∼92 miRNAs appear as key mediators to appropriately tune PI3K activity. On the other hand, in lymphoid tumors overexpression of the miR-17∼92 miRNAs is a common oncogenic event. In this review, we touch on what we have learned so far about the miR-17∼92 miRNAs, particularly with respect to their role in lymphocyte development, homeostasis and pathology.

1. Introduction

During the last 20 years, the concept of RNA as a passive messenger or structural component was extended to that of RNA as a central regulatory entity of gene expression. Non-protein coding RNAs, including microRNAs (miRNAs), have deterministic roles in virtually all physiological and pathological processes [1]. Primary miRNA transcripts (pri-miRNAs) are generated by RNA polymerase II [2–4]. Pri-miRNAs fold back on themself to form a substrate for the microprocessor complex containing the RNase III Drosha whose nucleolytic activity liberates one or multiple ∼60 nucleotide stem-loop precursor miRNAs (pre-miRNAs) [5,6]. Pre-miRNAs are exported into the cytoplasm by Exportin 5 and RAN-GTP [7–9] and further processed into mature miRNA duplexes by the RNase III Dicer [10,11]. After loading onto the binding pocket of an Argonaute (Ago) protein, thermodynamic properties specific to each duplex result in the ejection and degradation of one strand [12]. The remaining single-stranded mature miRNA (∼22 nucleotides) guides the ribonucleoprotein complex to partially complementary sequences on messenger RNAs (mRNA). The specificity of miRNA:mRNA Watson-Crick pairing is determined by the miRNA seed region (i.e. nucleotides 2–7 at the 5′ end), which exhibits perfect complementarity with the corresponding seed-match region in the target mRNA [13]. Base pairing outside of the seed region is imperfect. Of note, “seedless” miRNA:mRNA interactions have been described, but their regulatory power seems limited [14,15]. miRNA:mRNA interactions cause translational repression and/or mRNA destabilization. Hence, the biological function of a miRNA is largely restricted by its mRNA-interactome. Recently discovered nuclear functions of mature miRNAs, i.e. regulation of transcriptional activity by Watson-Crick pairing with cognate sequences on DNA, have been reviewed elsewhere [16].

1.1. miRNAs in action

By virtue of their small size and imperfect base pairing, individual miRNAs may interact with hundreds of seed-matches across the transcriptome. Furthermore, an estimated 70% of all human protein-coding genes are computationally predicted miRNA targets, a majority containing multiple binding sites [17,18]. Deletion of key processing enzymes of miRNA biogenesis in mice resulted in devastating phenotypes

Abbreviations: BCR, B-cell receptor; Ago, Argonaute; Ig, Immunoglobulin; RAG, recombination activating gene; Bcl2, B-cell lymphoma 2; Bim, Bcl2-interacting mediator of cell death; PI3K, phosphoinositide 3-kinase; Foxo1, forkhead box O1; Pten, Phosphatase and Tensin Homolog; Phlpp2, PH Domain and Leucine Rich Repeat Protein Phosphatase 2; GC, Germinal center; S1PR1, Sphingosine 1-phosphate receptor 1; SLE, Systemic Lupus Erythematosus; TCR, T-cell receptor; MHC, Major Histocompatibility Complex; Treg, regulatory T-cell; LCMV, Lymphocytic Choriomeningitis Virus; TgfbR2, Transforming Growth Factor Beta Receptor II; Creb1, cAMP Responsive Element Binding Protein 1; BL, Burkitt's lymphoma; DLBCL, Diffuse Large B-cell Lymphoma; CLL, Chronic Lymphocytic Leukemia

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and the deregulation of thousands of mRNAs [19]. For example, in the hematopoietic system acute loss of Dicer resulted in the apoptotic loss of functional stem cells [20]. Individual miRNAs can indeed affect the mean expression of many targeted proteins, but mostly do so in a modest dose range [21,22]. This has led to the perception that single miRNAs, rather than substantially regulating "key targets", fine-tune protein levels in "gene regulatory networks" by targeting multiple genes simultaneously. However, salient case studies have confirmed the functional prominence of individual miRNA:mRNA interactions in specific cellular contexts [23–26]. In humans and mice, 2-fold dose changes of proteins by inactivation of one allele can cause pathologies [27]. mir-150 regulates the abundance of the transcription factor c-Myb over a 2-fold range in murine B cells, and this suffices for altered B-cell physiology upon miR-150 deletion [28].

1.2. Genomic organization of miRNAs

miRNAs are commonly categorized into seed families, groups of miRNAs that share substantial sequence similarities [15]. The members of one miRNA seed family are likely to redundantly repress a set of common target mRNAs and hence share biological functions.

Pri-miRNAs can be expressed from independent transcription units, or from introns or exons of protein-coding genes [29]. Unlike mammalian protein coding genes, up to 60% of miRNA genes are located within close proximity (< 10 kb) and organized in clusters of two or more miRNAs [30]. Regardless, each cluster gives rise to one polycistronic transcript, and all miRNAs generated from one cluster are under the same transcriptional control. The structural analogy to prokaryotic operons has led to the speculation that co-transcribed miRNAs from different seed-families target functionally related genes. Hence, clustered miRNAs may offer an increase in miRNA gene dosage, expanding the capacity of functional target repression.

2. The miR-17 ~ 92 cluster and its paralogues

miR-17 ~ 92 is a gene that exemplifies the complexity associated with miRNAs. The miR-17 ~ 92 cluster maps to an 800 base-pair region in the human genomic region *C13orf25*, and its miRNAs are generated from a 7 kb transcript encoded by the non-protein coding gene *MIR17HG*. Based on seed sequence homology, the miR-17 ~ 92 miRNAs fall into four families: miR-17 (miR-17-5p and miR-20a), miR-18, miR-19 (miR-19a and 19b-1) and miR-92 (miR-92-1) [31]. Both, the sequences of the intra-cluster miRNAs and the polycistronic gene structure are highly conserved throughout vertebrates [32].

A process of duplication in early vertebrate evolution has given rise to two paralogue clusters, *miR-106a ~ 363* and *miR-106b ~ 25,* that are located on human chromosome X and 7, respectively [30,33]. *miR-17 ~ 92* and *miR-106b ~ 25* are abundantly expressed across many tissues and cell types, while *miR-106a ~ 363* expression is negligible. Together, these three clusters comprise 15 miRNA stem-loops (6xmiR-17 family; 2xmiR-18 family; 3xmiR-19 family; 4xmiR-92 family).

Whereas many miRNAs are expressed with high spatiotemporal specificity, the miR-17 ~ 92 miRNAs are among the most abundant miRNAs throughout tissues, with highest levels in less differentiated cells [33,34]. As functional miRNA-mediated repression requires high miRNA levels [35,36], it is likely that miR-17 ~ 92 represses mRNAs in various contexts. Germline deletion of the miR-17 ~ 92 cluster in mice caused perinatal lethality and accompanying lung, skeletal, cardiac and immune defects [33]. Conditional knockout mice revealed further roles in neural stem cell biology and kidney development and function [37–39]. In one-third of Feingold syndrome patients, haploinsufficiency of the miR-17 ~ 92 locus has been proposed to be causative for skeletal abnormalities, mirroring some of the defects observed in knockout mice [40].

3. miR-17 ~ 92 in B cells

3.1. Early B-cell development

Developmental progression of B cells from uncommitted hematopoietic progenitors to their functional endpoints, antibody-secreting plasma cells, encompasses a continuum of transit amplifying stages. In the bone marrow, sequential V(D)J recombination at immunoglobulin (Ig) heavy and light chain gene loci - catalyzed by recombination activating gene 1 (RAG1) and RAG2 - results in the generation of immature B cells that carry a fully assembled and unique BCR (B cell receptor) of the IgM isotype.

Although the miR-17 ~ 92 miRNAs are abundantly expressed in all B-cell subsets, their levels decrease two-to threefold on maturation [34,41].

Mice lacking enzymes specifically in B cells that are crucial for miRNA-biogenesis yielded a (near-)complete block in early B-cell development at the pro-B to pre-B transition [42–44]. This phenotype is likely caused by combined defects in differentiation, proliferation and apoptosis, as concomitant overexpression of the pro-survival Bcl2 (B-cell lymphoma 2) protein or deletion of the pro-apoptotic Bim [45,46] (Bcl2-interacting mediator of cell death) protein caused an accumulation of mature B cells despite breaking the developmental block. Eliminating miR-17 ~ 92-expression in early B cells either alone or in combination with its paralogue clusters phenocopied this phenotype [47]. However, whereas pro-B cell apoptosis in chimeric mice harboring a miR-17 ~ 92-deficient immune system is associated with increased Bim levels [33], conditional ablation of miR-17 ~ 92 specifically in early B cells did not cause Bim up-regulation [47]. Accordingly, the developmental block could not be rescued by ectopically expressing Bcl2. Restoring the expression of miR-17 ~ 92 miRNAs, either individually or in combination, revealed a central role for miR-17 in early B-cell development. This article is first to assess regulatory functions of single miRNAs from the miR-17 ~ 92 in B cell hematopoiesis. Yet, heterozygous and homozygous deletions of the two putative targets *Pten* (Phosphatase and Tensin Homolog) and *Phlpp2* (PH Domain and Leucine Rich Repeat Protein Phosphatase 2) in miR-17 ~ 92-deficient cells failed to revert the developmental block. As deletion of the cluster in this mouse model occurs early in the hematopoietic lineage, it is possible that loss of miR-17 ~ 92 affects also commitment and differentiation into the B lineage, which could not be rescued by *Pten* or *Phlpp2* deletions. Thus, although repression of Bim, Pten or Phlpp2 might provide survival signals to pro-B cells, miR-17 ~ 92 targets that are involved in B-cell differentiation remain to be identified.

3.2. B-cell tolerance

From the immature stage on, B-cell decision-making depends on signals generated by the BCR and associated co-receptors [48,49]. The stochastic nature of V(D)J recombination during early B-cell development causes the appearance of B cells that carry autoreactive BCRs. Autoreactive BCR-signaling initiates apoptosis (clonal deletion) in a process termed negative selection. Alternatively, these cells can avoid elimination by continuing V(D)J recombination (receptor editing). Proper BCR-dependent but probably ligand-independent signals are required for the survival and maturation of functional and non-autoreactive B cells (positive selection) [50]. Balanced phosphoinositide 3-kinase (PI3K)-activity downstream of the BCR and its co-receptors, notably antagonized by the phosphatase Pten [51], is key for functional B-cell selection and survival [49].

The miR-17 ~ 92 miRNAs emerged as a central mechanism by which the PI3K pathway positively controls its own activity via repression of Pten in an auto-stimulatory loop, aiding positive selection [52] (Fig. 1). PI3K signaling promotes transcriptional up-regulation of miR-17 ~ 92 via Myc and consequently miR-17 ~ 92-dependent repression of Pten (Myc/miR17 ~ 92/Pten loop). Deficiency of CD19 – a BCR co-receptor –

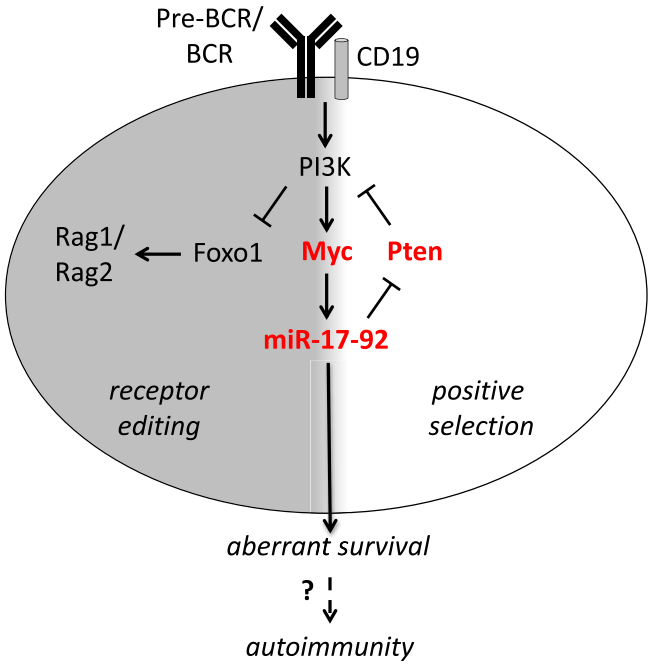

Fig. 1. The autostimulatory Myc/miR17∼92/Pten loop tunes PI3K-signaling downstream of the BCR and controls B-cell selection and maturation. PI3K-activity is facilitated by an autostimulatory Myc/miR17∼92/Pten loop, where enhanced expression of miR-17∼92 causes the repression of Pten, a negative regulator of PI3K-signaling. Altered expression of Myc, miR17-92 or Pten modulates PI3K activity and may pose a risk for aberrant survival and autoimmunity.

caused apoptosis of immature B cells in mice due to diminished PI3K-signaling [53]. Ectopic expression of miR-17∼92 reconstituted B-cell development in these mice, providing evidence that miRNAs, by altering intracellular biochemical pathways, are capable of restoring severe developmental impairments that result from the deficiency of one key regulator [52]. Mechanistically, CD19-deficient B cells display decreased levels of the Myc protein, resulting in diminished miR-19 expression and consequently an increase in Pten protein. Notably, PI3K activity downstream of the precursor BCR in pre-B cells reduces RAG levels during light chain rearrangement through indirect miRNA-mediated repression of forkhead box O1 (Foxo1) [43]. It is a possible scenario that PI3K-signaling would block ongoing V(D)J arrangements and allow for developmental progression via miR-17∼92 function (Fig. 1). Indeed, we could show that the Myc/miR17∼92/Pten axis tunes PI3K activity to control the expression of RAG proteins by impacting Foxo1 levels [54].

Ectopic expression of miR-17∼92 or miR-19 alone in the IgM^b-macroself mouse model of central tolerance [55] rescued the development and maturation of autoreactive B cells, and allowed for receptor editing [47]. Pten is rate-limiting in this model as its overexpression caused the survival and maturation of autoreactive B cells in IgM^b-macroself mice.

These results reveal a prominent role for the miR-19 family miRNAs and their mRNA target Pten in B-cell central tolerance.

3.3. Mature B cells

The majority of naïve mature B cells are of the B2 type. Deletion of miR-17∼92 late during B-cell maturation caused a moderate reduction in peripheral B-cell numbers and increased Bim protein in these cells, indicating a function for the cluster in the homeostasis of the mature B2-cell pool [56]. Innate-like B1 B cells are a major source of polyreactive (and frequently self-reactive) antibodies. Overexpression of miR-17∼92 caused an accumulation of the B1a subset [57]. Conversely, deletion of miR-17∼92 in mature B-cells led to a reduction in B1-cell numbers and defective antibody responses in these cells [56].

3.4. Activated B2 cells

Once activated by antigen, mature B cells can be recruited into anatomically distinct sites in lymphoid organs termed germinal centers (GC). In GCs, B cells undergo iterative rounds of divisions and somatic mutations of their rearranged Ig variable (V) region genes to improve the affinity of their BCR. GC B cells undergo class-switch recombination involving the IgH constant region genes, hence permitting the production of multiple functional Ig isotypes. Most GC-derived B cells become short-lived antibody-secreting plasmablasts, but some differentiate into long-lived bone marrow-resident antibody-secreting plasma cells or migratory memory B cells.

Contrasting B-cell development, relatively little is known about the role of miR-17∼92 in antigen-activated B cells. Deletion of miR-17∼92 late during B-cell development resulted in increased plasma cell homing to the bone marrow after immunization [56]. Sphingosine 1-phosphate receptor 1 (S1PR1), a key molecule in immune cell migration to the bone marrow, was identified as a target of miR-17∼92 in this process [56]. In addition, CSR to IgG2a/c was impaired as a consequence of the up-regulated transcription factor Ikaros, a miR-92 target. This result could be of relevance for autoimmune diseases, as high serum IgG levels are found in systemic lupus erythematosus (SLE) patients. Finally, mature B-cell specific ablation of miR-17∼92 in a mouse lupus model reduced IgG2c production and alleviated pathology [56]. These results suggest a therapeutic potential of targeting the miR-17∼92 miRNAs in SLE.

4. miR-17∼92 in T cells

4.1. T-cell development

After seeding from the bone marrow, thymic progenitors progress through a series of developmental stages until expressing a rearranged TCR (T-cell receptor) through gene recombination catalyzed by RAG1 and RAG2. In early thymocytes, survival signals provided by growth factors such as IL-7 prevent apoptosis and promote differentiation. Thymocytes undergo positive selection based on the ability of their TCR to recognize peptide-self MHC (major histocompatibility complex) molecules displayed by the surrounding thymic cells. Conversely, maturing thymocytes that receive autoreactive TCR signals are eliminated during negative selection. Developmental death guarantees that fully mature $CD4^+$ or $CD8^+$ T cells that finally emerge into the periphery are functional and suitably tolerant to self.

The initial description of chimeric mice harboring a miR-17∼92-deficient immune system revealed no obvious gross phenotypes in T-cell development [33]. However, a more detailed analysis of a conditional mouse model lacking the miR-17∼92 miRNAs only in immune cells revealed reduced IL-7Ra expression and limited responsiveness of pre-thymic T-cell progenitors to IL-7 [58]. Consequently, miR-17∼92 deficient thymocytes did not efficiently contribute to T-cell development in competitive bone marrow chimeras.

4.2. Activated T cells

Differentiation of mature naïve $CD4^+$ or $CD8^+$ T cells into memory, effector or regulatory T (Treg) cells is initiated upon interaction of the TCR with cognate antigen on antigen-presenting cells, such as dendritic cells.

Whereas the miR-17∼92 miRNAs are progressively down-regulated during T-cell differentiation, they are strongly induced upon activation in a CD28-dependent manner [59], and finally down-regulated during terminal differentiation into memory cells [60]. Mice where miR-17∼92-expression was genetically eliminated specifically in activated

$CD8^+$ T cells showed impaired expansion of effector $CD8^+$ T cells upon infection with lymphocytic choriomeningitis virus (LCMV). In contrast, overexpression of the cluster promotes a skewing towards short-lived effector cells. Mechanistically, miR-17∼92 promotes mTOR-signaling and repression of Pten in $CD8^+$ effector T cells [60,61].

Naive $CD4^+$ T cells can differentiate in response to polarizing cytokines into various T-cell subsets including Th1, Th2, Th17, and Treg cells. Ectopic expression of miR-17∼92 miRNAs skews polarization towards a Th1 or Th17 phenotype at the expense of Th2 and Tregs [62]. miR-19b and miR-17 have been identified as the essential cluster members in Th1 polarization, with Pten, transforming growth factor beta receptor II (TgfbR2) and cAMP responsive element binding protein 1 (Creb1) as major mRNA targets. Deletion of miR-17∼92 in differentiating Tregs in vivo resulted in reduced Treg numbers, presumably due to impaired proliferation and increased apoptosis [63], and in exacerbated experimental autoimmune encephalitis [59]. Specifically, miR-17∼92-expression in Tregs was required for the production of IL-10. Ectopic expression of miR-17∼92 in lymphocytes promotes T-cell proliferation and reduces apoptosis, hence causing lymphoproliferation and fatal Ig-mediated autoimmunity [57]. The skewed CD4/CD8 ratio in these mice implies impaired negative selection.

T_{FH} cells are specialized $CD4^+$ antigen-activated cells that provide survival signals to antigen-specific GC B cells. Specific deletion of miR-17∼92 in $CD4^+$ T cells resulted in reduced T_{FH} cell numbers in a viral infection model, and conversely $CD4^+$ T-cell-specific overexpression of the cluster increased the numbers of both, $CD4^+$ T cells and GC B cells [64,65]. Pten, RORα and Phlpp2 were identified as key targets. Haploinsufficiency of *Pten* partially rescued T_{FH} cell numbers when miR-17∼92 was absent in T cells, and haploinsufficiency of *Rorα* restored the expression of T_{FH}-associated genes [64,65].

Recent studies uncovered that miR-17∼92 is required for donor Th1 and Th17 cells to mediate graft-versus-host disease as a consequence of allogeneic bone marrow transplantation in mice [66,67]. In contrast, graft-versus-leukemia activity remained unimpaired. Pharmacological inhibition of either miR-17 or miR-19 with antagomirs alleviated the disease and prolonged the survival of recipient mice.

5. miR-17∼92 in lymphocyte malignancy

C13orf25, the genomic region coding for miR-17∼92, is commonly amplified in B-cell lymphomas/leukemias such as Burkitt's lymphoma [68,69], diffuse large B-cell lymphoma (DLBCL) [31,70–76], primary cutaneous B-cell lymphoma [77], Hodgkin lymphoma [78], mantle cell lymphoma [79], chronic lymphocytic leukemia (CLL) [80–82] and acute lymphoblastic leukemia [80,83], as well as multiple myeloma [84,85] and anaplastic large T-cell lymphoma [86] (Table 1). Retroviral mutagenesis screens identified the genomic region coding for miR-17∼92 as a common viral integration site associated with insertional activation or amplification [87,88]. One of the initial findings concerning miR-17∼92 was that Myc directly initiates pri-miR-17∼92 transcription [89,90]. Myc, an oncogene that is commonly overexpressed in B-cell lymphomas, robustly drives miR-17∼92 transcription in lymphomas [70,91]. Ectopic expression of miR-17∼92 in the Eμ-MYC lymphoma mouse model [92] shortens the latency for tumor onset and promotes tumor invasiveness [70,93]. Hence, miR-17∼92 was coined as the first “oncomiR”. Acute knockout of the cluster in Eμ-MYC lymphoma cell lines increased apoptosis and compromised transplantability into nude mice [94]. In models of acute lymphoblastic leukemia, acute deletion of the oncogenic drivers MYC or RAS, but not BCR-ABL, caused tumor cell apoptosis that was associated with a decrease in all miR-17∼92 but miR-92 [95]. In line, B-cell specific deletion of miR-17∼92 in the λ-MYC lymphoma model only allowed the outgrowth of tumors that had avoided miR-17∼92 loss [96]. Independent studies revealed that the miR-19 family miRNAs are essential and sufficient for the transformation of MYC-expressing B cells [94,97,98]. Therefore, miR-19 family miRNAs might be a prime target for anti-cancer therapeutics.

Aberrant MYC expression promotes excessive proliferation, yet also evokes potent induction of apoptosis [93]. The MYC-driven transcription factors of the E2F family (E2F1-3) play a central role in G1-to-S phase progression [99], and have been shown to directly promote miR-17∼92 transcription [90,100,101]. As the E2F1-3 mRNAs are also targets of the miR-17 family miRNAs, these molecules form an autoregulatory loop [100,101]. MYC-driven murine lymphomas are addicted to miR-17∼92, and this was speculated to be partially due to its repressive effect on Bim and the chromatin regulatory genes *Sin3b*, *Hbp1*, *Suv420h1* and *Btg1* [102]. Knockdown of Pten in miR-17∼92-deficient lymphoma cells reduced apoptosis in vitro and enhanced tumor formation in vivo [94]. Notably, PI3K-signaling synergizes with Myc to promote lymphomagenesis [103], suggesting that the Myc/miR17∼92/Pten loop (Fig. 1) may also play a role in lymphocyte malignancies. Recently, three groups uncovered a role as *bona fide* oncogene for miR-17∼92 in murine B-cell lymphoma models [96,104,105]. Two negative regulators of PI3K-signaling, Pten and Phlpp2, and the NFkB deubiquitinases A20 and Cyld have been proposed as targets [96]. Of note, miR-17∼92 was implicated in DLBCL-tumor cell survival by directly repressing PTPROt and PP2A – mediators of SYK-pathway activity downstream of BCR signaling [106]. This result is of interest because a majority of B-cell lymphomas depend on BCR signaling for their survival.Whereas much is known about the oncogenic role of miR-17∼92 in B cells, only little information is available about T-cell malignancies. miR-19 and Notch1 were reported to cooperate in promoting T-cell acute lymphoblastic leukemia in mice [107]. In anaplastic large T-cell lymphoma in humans, STAT3 was identified as a direct transcriptional activator of miR-17∼92 [108].

6. Regulation of miR-17∼92 abundance and activity

6.1. Differential processing of the miR-17∼92 miRNAs

Although transcribed as a single unit, differential processing allows the uncoupling of the production of individual mature miRNAs from within one pri-miRNA. In hematopoietic pathologies, the relative abundance of miR-17∼92 miRNAs varies substantially between disease states and tumor types, and is significantly different from the healthy cells of origin (Table 1). In asthma pathogenesis, miR-19a expression is disproportionally high in airway T cells [109]. Peripheral B cells from atherosclerosis patients specifically up-regulate miR-19a, a finding that correlates with decreased IL-10 expression in these cells [110]. In germinal center DLBCL, miR-17 and miR-19 family members are most abundant, but a different subset of DLBCL, primary mediastinal lymphoma expresses high levels of miR-92a [74]. In multiple myeloma patients, poor prognosis correlates with an increased relative abundance of miR-17 and miR-92a family miRNAs [111].

Differential expression of miR-17∼92 miRNAs in pathologic conditions versus their healthy cells of origin could simply be a bystander effect of disease/transformation histories specific to each pathology. Alternatively, unbalanced expression might provide a selective advantage for the pathogenic process by shifting the functional target set of the cluster. Arguing for the latter, retroviral-mediated miR-19 overexpression in murine hematopoietic stem cells led to B-cell hyperplasia, whereas overexpression of miR-92a caused erythroleukemia [82]. Co-expression of miR-17 along with miR-92a abrogated leukemia, presumably by targeting genes that regulate erythroid proliferation and survival. In CLL patients with aggressive clinical outcome, loss of p53 function correlates with reduced levels of miR-17 miRNAs and increased expression of miR-19 and miR-92a miRNAs. Finally, the authors suggest that the imbalanced expression of miR-17∼92 miRNAs might be a direct consequence of p53 loss-of-function and causative for transformation. In CLL patient samples, high miR-19a expression correlates with low levels of PTEN [112], suggesting a potential role for miR-19:Pten interactions.

Table 1
Alterations of miR-17 ~ 92 abundance in human lymphomas.

Pathology	miR-17-92	Function	Ref.
B-cell Lymphoma			
Multiple Myeloma (MM)	miR-17; miR-20; miR-92a	High levels of miR-17, miR-20 and miR-92a are associated with shorter progression-free survival	[83]
	miR-20	miR-20 is elevated in plasma of MM patients	[84]
Chronic Lymphocytic Leukemia (CLL)	miR-17 ~ 92	All cluster members are moderately up-regulated	[79]
	miR-92a-3p	Overall decrease of miR-92a-3p expression in CLL cells; low levels associate with a poor prognosis	[80]
	miR-17 ~ 92	Relative increase in miR-19b and miR-92a; relative decrease in miR-17 and miR-20	[81]
Acute Lymphoblastic Leukemia (ALL)	miR-17 ~ 92	All cluster members are moderately up-regulated	[79]
	miR-17 ~ 92	all miR-17 ~ 92 miRNAs are down-regulated in BCR-ABL$^+$ ALL, but not in BCR-ABL$^-$ ALL	[82]
Primary Cutaneous B-cell Lymphoma	miR-20	miR-20 is overexpressed in tumor cells; high levels of miR-20 are associated with poor prognosis	[76]
Diffuse Large B-cell Lymphoma (DLBCL)	miR-17 ~ 92	miR-18, miR-19b and miR-20 are the most up-regulated miR-17 ~ 92 miRNAs in DLBCL as compared to non-tumor B cells and follicular lymphoma cells	[71]
	miR-17-92	Amplification of the cluster in GC-DLBCL	[70]
	pri-miR-17 ~ 92	Pri-miR-17 ~ 92 is overexpressed in DLBCL; overexpression associates with shorter progression-free survival and overall survival	[72]
	miR-92a	Overexpression of miR-92a in primary mediastinal large B-cell lymphoma as compared to DLBCL and classical Hodgkin lymphoma	[73]
	miR-92a	Plasma miR-92a levels significantly decreased in patients as compared to healthy controls	[74]
	miR-17; miR-18; miR-19a	High levels of miR-17, miR-18 and miR-19a in AIDS-related DLBCL and Burkitt's lymphoma	[75]
	Pri-miR-17 ~ 92	On average tenfold increase of pri-miR-17 ~ 92 in DLBCL as compared to control cells	[69]
	Pri-miR-17 ~ 92	First description of amplification of the cluster in tumors as determined by comparative genomic hybridization	[31]
Burkitt's Lymphoma	miR-17 ~ 92	All cluster members are differentially expressed, with miR-19a reaching the highest levels; high miR-17 levels correlate with poor overall survival	[67]
	miR-17 ~ 92	Amplification of the cluster common and mutually exclusive with deletion of E2F2	[68]
Hodgkin Lymphoma	miR-17 ~ 92	All cluster members are up-regulated compared to other B-cell lymphoma types	[77]
Mantle Cell Lymphoma	miR-17 ~ 92	High levels of miR-19a and miR-92a associate with more aggressive disease; high levels of miR-17 and miR-18 associate with less aggressive disease	[78]
T-cell Lymphoma			
Anaplastic Large T-cell Lymphoma	miR-17; miR-20	miR-17 and miR-20 are higher expressed in ALK$^+$ as compared to ALK$^-$ lymphomas	[85]

6.2. First clues towards an understanding of differential processing of the miR-17 ~ 92 miRNAs

Several microprocessor- and Dicer-associated factors that facilitate or inhibit miRNA maturation have been identified. However, there is still surprisingly little information on the molecular pathways involved in the processing of pri- and pre-miRNAs. One determinant of the relative abundance of mature miR-17 ~ 92 miRNAs is the pri-miR-17 ~ 92 tertiary structure. Whereas miR-92a is immediately processed by microprocessor, cis-acting sequences within pri-miR-17 ~ 92 cause an inhibitory RNA conformation for other cluster members. This repression can be relieved by cleavage through the endonuclease rCPSF3 and the spliceosome-associated ISY1 hence allowing for processing by microprocessor [113]. Tertiary interactions within the intact pri-miR-17 ~ 92 limit the accessibility of the miR-92a hairpin. Adding an additional regulatory step, these results challenge the traditional two-step processing model. This is also reflected by a common relative depletion of miR-92a as compared to most other miR-17 ~ 92 miRNAs. Notably, RNA-binding proteins have been shown to interact with individual pre-miRNA components of miR-17 ~ 92. For example, the processing of miR-18 by Drosha is facilitated by its binding to hnRNPA1 [114].

Finally, relative miR-17 ~ 92-abundance does not necessarily reflect biological activity. Developing methodologies that allow to faithfully compare the abundance of endogenous mature miRNAs with the fraction of actively mRNA-targeting (i.e. RISC-loaded) miRNAs will be critical to clarify this issue.

6.3. Interactions among miR-17 ~ 92 components

Although individual members of heterogeneous miRNA clusters might share an overlapping mRNA-targetome, our knowledge on this topic is strikingly descriptive.

Selective deletion of individual miR-17 ~ 92 miRNAs in mice demonstrated both, functional cooperation and specialization among miR-17 ~ 92 seed family miRNAs. Whereas miR-19 function dominates in the formation of MYC-driven lymphomas with a possible accessory role for miR-17, miR-17 has a dominant role in early B-cell development, with miR-19 and miR-92a contributing [47,98]. B-cell central tolerance is mainly regulated by miR-19, with accessory roles for miR-17 [47,98]. In contrast, most developmental defects in miR-17 ~ 92-deficient embryos depend on the loss of functional cooperation between multiple miR-17 ~ 92 seed families [98]. Overexpression of miR-19a or miR-92a in hematopoietic stem cells resulted in two different outcomes, B-cell hyperplasia or erythroleukemia, respectively [82]. The fact that miR-92a-induced erythroleukemia was abrogated when co-overexpressing miR-17 suggests that also functional antagonism might exist among cluster members. Another example of functional antagonism is that miR-92a negatively regulates the oncogenic cooperation of the cluster with Myc [93]. miR-92a represses Fbw7 and causes the stabilization of Myc, excessive proliferation and p53-dependent apoptosis. Consequently, miR-92a antagonizes the oncogenic effect of miR-19. However, this mechanism might be lost during B-cell transformation as the lymphoma cells tested favored an increase of miR-19 over miR-92a.

These results illustrate the dynamic functional interactions between individual members of miR-17 ~ 92, however, how this is achieved mechanistically at the level of their mRNA-targetome remains elusive.

7. Future directions

Over the past decade it has become clear that the miR-17 ~ 92 cluster miRNAs substantially contribute to adaptive immunity and associated pathologies. Contrasting the prominent phenotypes observed in miR-17 ~ 92-deficient mice, the set of validated targets is strikingly small. Whereas Pten has been confirmed as a key target during B-cell selection, suspected direct targets in B-cell development are still in doubt and may not be functional in this specific cellular context [47]. Further studies identifying additional target genes that may control commitment and differentiation along the hematopoietic program are warranted. Along with a recent publication that compares the miR-155:mRNA interactome between different immune cell types [115], these results suggest a high degree of context-dependent miRNA-mediated gene regulation, where the context may include but is not

limited to the expression and cellular localization of miRNAs and their target mRNAs, the stability of miRNA:mRNA interactions, the accessibility and mRNA modifications of individual binding sites, the mRNA tertiary structure and interactome with RNA binding proteins. Associated with the plasticity of these contextual clues during development, differentiation and pathology, functional miRNA:mRNA interactions in a given context in vivo are likely to be difficult to assess in vitro. This is particularly relevant for lymphocyte development and selection, as these processes cannot be recapitulated in culture systems yet. Notably, the functional targetome of miRNA clusters might substantially shift as a result of differential processing of the individual miRNAs. Differential abundance of the miR-17∼92 miRNAs has been repeatedly described in lymphomas. It is well conceivable that intra-cluster differential expression might also play a role in developmental transitions and selection processes of lymphocytes. Hence, to ultimately confirm and study the biologically most relevant miRNA:mRNA interactions, mutagenesis of individual binding sites in endogenous physiological or pathological contexts in combination with differential biochemical mapping of binding sites will be required.

Conflicts of interest

The authors declare that they have no competing interests.

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