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microRNA Fine-Tuning of the Germinal Center Response

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Germinal centers (GCs) are complex multicellular structures in which antigen-specific B cells undergo the molecular remodeling that enables the generation of high-affinity antibodies and the differentiation programs that lead to the generation of plasma–antibody-secreting cells and memory B cells. These reactions are tightly controlled by a variety of mechanisms, including the post-transcriptional control of gene expression by microRNAs (miRNAs). Through the development of animal models with B cell-specific modified miRNA expression, we have contributed to the understanding of the role of miRNAs in the regulation of GC responses and in B cell neoplasia. Here, we review recent advances in the understanding of the role of miRNAs in the regulation of B cell and T follicular helper physiology during the GC response and in the diseases associated to GC response dysregulation.

Keywords: microRNA, germinal center, antibodies, autoimmunity, neoplasia

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INTRODUCTION

The germinal center (GC) response is a key B lymphocyte maturation and differentiation program essential for the generation of competent protective immunity. The GC response is initiated in mature B lymphocytes after antigen encounter and leads to the generation of memory B cells and plasma antibody-secreting cells that produce antibodies with high antigen affinity and with different immunoglobulin (Ig) isotypes, conferring the Ig molecule with the ability to orchestrate different immune effector responses (1, 2). At the molecular level, these reactions are initiated by the activity of activation induced deaminase (AID), an enzyme that deaminates cytosines in the Ig genes, triggering somatic hypermutation (SHM) and class switch recombination (CSR), processes respectively responsible for the changes in affinity and isotype in the Ig genes. At the cellular level, initiation of the GC reaction requires the cognate interaction of antigen-activated B-lymphocytes with a specialized subset of GC T CD4 cells, the follicular T helper (Tfh) cells. Tfh-GC B cell interactions are dependent on a number of molecule interactions that signal for full B and Tfh cell differentiation together with cellular localization in follicles. These interactions include T-cell receptor recognition of B cell peptide-MHC complexes as well as CD40 and ICOS ligand co-receptor interactions (3). Developing Tfh and B GC cells are influenced by changing cytokine, chemokine and cellular environments through the induction of specific transcriptional programs (4). Gene transcription in Tfh and B cells is regulated by key GC transcription factors such as BCL6, as well as by RNA-binding proteins and microRNAs (miRNAs) (3, 5). miRNAs are small non-coding RNA molecules that drive post-transcriptional negative regulation of gene expression by

promoting the degradation or translational blockade of partially complementary target mRNAs. Mature miRNAs are 21-24-nucleotide RNA molecules processed from longer RNA precursors in two consecutive cleavage steps mediated by the RNase III enzymes Drosha and Dicer (6). Ablation of miRNAs in miRNA-processing-enzyme deletion knockout models has demonstrated that miRNAs play essential roles in diverse developmental, cellular, and physiological processes (7, 8). miRNAs fine-tune cellular gene expression networks and have emerged as essential regulators of GC differentiation responses.

miRNAs IN PHYSIOLOGICAL GC REGULATION

Studies of global miRNA depletion in GC B and T cell-specific models showed that miRNAs are essential for proper GC formation (9, 10). Dicer-mediated miRNA depletion after AID expression in early activated GC B cells impaired the production of high-affinity class-switched antibodies and the generation of memory B and long-lived plasma cells after T cell-dependent immunization due to defects in B cell proliferation and survival (9). Likewise, DGCR8-Drosha complex-mediated miRNA depletion in CD4 T cells showed that CD4 T cell-expressed miRNAs are essential for the differentiation of Tfh cells and the induction of GC B cells during T cell-dependent immunizations (10). Interestingly, miRNAs are not only required to regulate Tfh and GC B cell function in a cell intrinsic manner, but are also important contact-independent mediators of T-B cellular communication (**Figure 1**). This communication occurs through the transfer to B cells of a restricted set of T cell-derived miRNAs in extracellular vesicles and modulates the efficiency of GC generation and antibody secretion in response to immunization (11).

miRNAs in the Regulation of B Cells in the GC

The most extensively studied GC B cell miRNA is miR-155, whose expression is upregulated after mature B cell activation and in GC B cells (12–15). Infection of miR-155-deficient mice with pathogenic bacteria showed that miR-155 expression is required to control pathogen-induced disease (16). Characterization of the response to T cell-dependent immunizations in miR-155$^{-/-}$ loss-of-function and miR-155^{KI} gain-of-function mouse models revealed that miR-155 expression is required for efficient adaptive immune responses, including the generation of GC B cells and the secretion of antigen-specific antibodies (12, 16). miR-155 is a positive regulator of the GC response, and deficiency in miR-155 expression leads to reduced cytokine production, IgG1 secretion, impaired affinity maturation, and plasmablast B cell generation in a B cell autonomous manner (12, 17, 18). miR-155 controls affinity-based selection, at least in part, by protecting light zone (LZ) GC c-MYC^{+} B cells from apoptosis (19).

Transcriptome studies showed that miR-155 regulates the expression of numerous mRNAs in B cells (17, 18), although the functional consequences of miR-155-dependent mRNA regulation in GC B cells has been characterized for only a few miR-155 targets. The transcription factor PU.1 is a direct miR-155 target implicated in miR-155 mediated effects on CSR (17). PU.1 is encoded by *Sfpi1*, and the consequences of disrupting miR-155–*Sfpi1* mRNA interaction *in vivo* were determined by generating knock-in mice with a mutation in the miR-155 recognition site in the *Sfpi1* mRNA 3'UTR. miR-155-mediated PU.1 post-transcriptional regulation was shown to be required for efficient terminal plasma B cell differentiation and antigen-specific immunoglobulin (Ig) secretion through the downregulation of *Pax5* expression and genes involved in adhesion and B-T cell interactions (20).

The other well characterized miR-155 target in GC B cells is activation-induced deaminase (AID), the enzyme responsible for the molecular remodeling of Igs in the GC. Knock-in mice with a disruption of the miR-155 recognition site in the *Aicda* mRNA 3'UTR demonstrated that miR-155 expression in GC B cells is needed to limit AID expression, allow proper affinity maturation, and restrict oncogenic AID-mediated MYC-IgH chromosomal translocations (21, 22). GC tolerance of DNA damage is multilayered and temporally regulated (23), and miR-155 expression is in turn limited by the expression of BCL6 (24, 25), an important transcriptional regulator and proto-oncogene that inhibits the DNA damage response in GC B cells (26). In addition, miR-155 negatively regulates the expression of *Socs1*, a P53 activator important for the DNA damage response (27). miR-155 thus plays a dual role in modulating the accumulation of DNA double-strand breaks (DSB) associated with the GC reaction, regulating P53 activity by controlling the expression levels of *Aicda* and *Socs1*.

AID expression is directly regulated in B cells by yet other miRNAs in B cells. miR-361 is another BCL6-downregulated miRNA that targets *Aicda*, presumably in light-zone GC B cells (25). miR-181b, which is highly expressed in mature resting B cells and whose expression diminishes upon B cell activation, targets *Aicda* directly through the binding of several partly complementary sequences found in its mRNA 3'UTR (28). Thus, AID levels are controlled by different miRNAs at different stages of B cell activation.

Another miRNA that positively regulates the GC response upon its induction during B cell activation and in GC B cells is miR-217. Using gain- and loss-of-function mouse models, we showed that miR-217 promotes the generation of GC B cells and increases the generation of class-switched antibodies and the frequency of somatic hypermutation in B cells. We found that miR-217 regulates a DNA damage response and repair gene network that stabilizes BCL6 expression in GC B cells (29). Thus, miR-217 downregulates a network of genes that sense and repair genotoxic events on DNA, which in turn can increase GC B cell tolerance to DNA damage in the context of AID activity, very much like BCL6. Notably, we found that miR-217 protects BCL6 from previously described genotoxic stress-induced degradation (23), suggesting that both molecules form part of the same network that renders GC cells permissive to genomic instability and prone to malignant transformation.

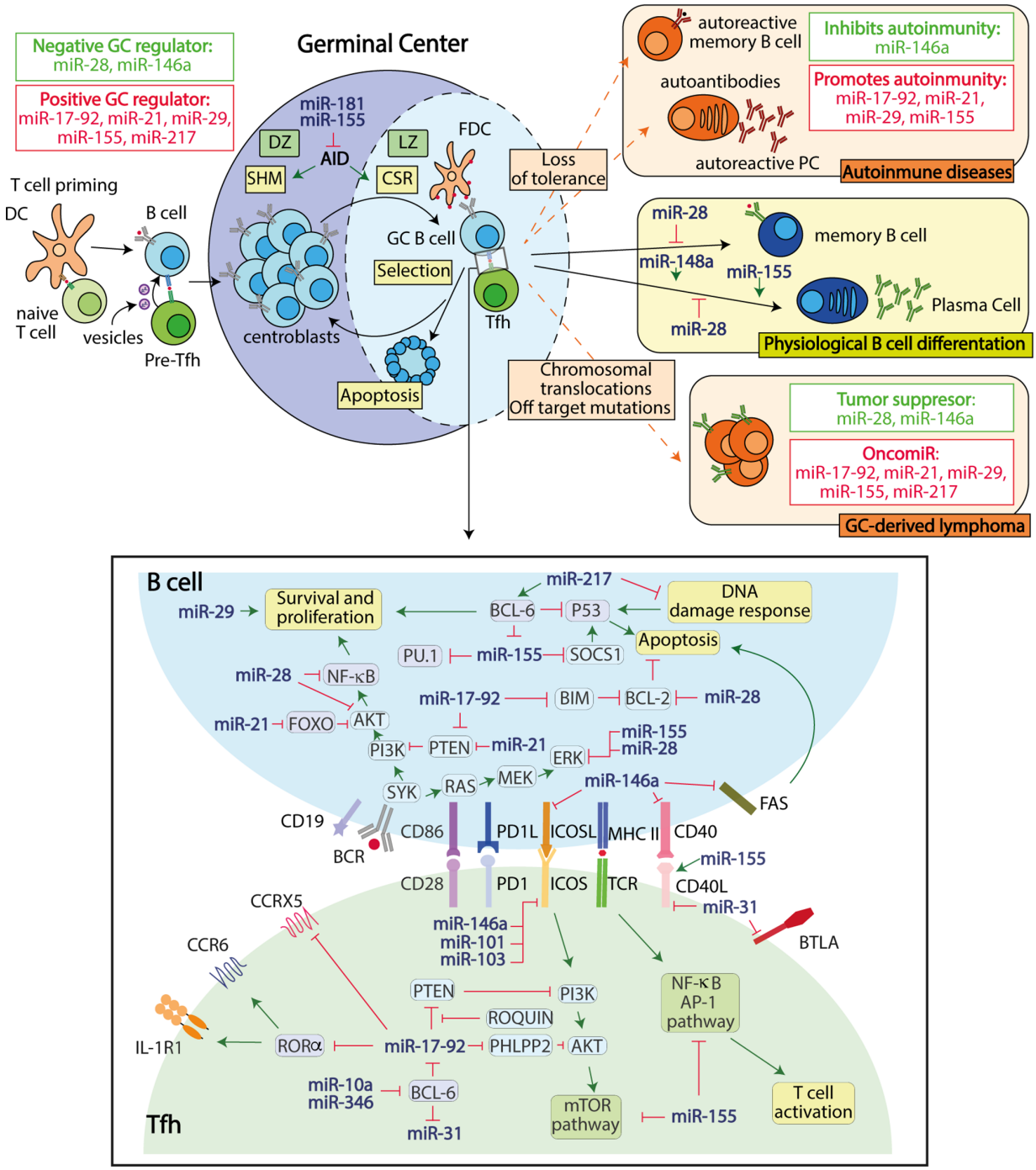

FIGURE 1 | miRNAs regulate gene expression in B and Tfh germinal center cells. Regulated miRNA expression is required to regulate B-Tfh cell interactions and ensure proper GC responses. GC-derived dysfunctions caused by miRNA alterations can lead to the development of autoimmunity and/or B cell neoplasia through the disruption of post-transcriptional control mechanisms required for the maintenance of GC homeostasis.

Positive regulation of terminal post-GC plasma B cell differentiation has been suggested to be regulated by other miRNAs. A likely candidate is miR-148a, the most abundant miRNA in human and murine plasma cells, which has been shown to promote plasma cell differentiation and survival *in vitro*. Importantly, miR-148a expression was shown to downregulate the expression of the GC transcription factors *Mitf* and *Bach2*, which block premature plasma cell maturation

and favor cell death (30). Definition of the role of miR-148a as a regulator of GC-dependent plasma cell differentiation *in vivo* would require the development of gain- or loss-of-function miR-148a B cell-specific mouse models.

GC miRNAs can also act as regulators that restrict the GC response, the best-characterized negative regulators of GC responses being miR-28 and miR-146a. miR-28 is a GC-specific miRNA (14, 15) whose expression is lost in numerous mature B-cell neoplasms (31–33). By combining gain- and loss-of-function approaches, we showed that miR-28 negatively regulates CSR and immunization-triggered GC and post-GC plasma and memory B cell generation. Combined transcriptome and proteome analysis upon inducible re-expression of miR-28 in B cells revealed that miR-28 expression induces the coordinated downregulation of the key BCR signaling gene network regulating B-cell proliferation and cell death (33), thus supporting the notion that miR-28 limits the strength of BCR signaling and regulates proliferation and survival of GC B cells.

miR-146a is expressed in B cells upon stimulation and within GC B cells (15), and loss of miR-146a causes a B cell-intrinsic increase in the GC response to immunization (34), spontaneous GC generation in aged mice, and increased production of anti-double–stranded DNA (dsDNA) auto-antibodies (35). miR-146a was shown to limit B cell GC functional responses by downregulating B cell expression of signaling pathway components involved in GC B Tfh cellular interactions, such as ICOSL (34) and CD40 (35).

Other miRNAs have also been suggested to negatively regulate terminal post-GC plasma and memory B cell differentiation. miR-125b, a miRNA highly expressed in dividing centroblasts in GC B cells (36), has been shown to inhibit plasma cell generation and antibody secretion *in vitro* (37, 38). Importantly, direct mRNA targeting by miR-125b was shown to downregulate the expression of BLIMP-1 and IRF-4 transcription factors, which are essential for plasma cell differentiation (36–39). *Prdm1*, the gene encoding BLIMP-1, is a direct target of other highly expressed GC B cell miRNAs, including miR-9, miR-30a, and let-7 family miRNAs (40–43). Interestingly, the expression of miR-30a and miR-125b is regulated epigenetically in B cells and can be modulated using histone deacetylase inhibitors to inhibit BLIMP-1 expression in the context of antibody responses and GC-derived diseases (44–46). Memory B cell generation is associated to changes in chromatin accessibility and miRNA expression, and miR-181 has been recently identified as a major gene expression regulator during memory B cell differentiation (47).

Overall, these studies have identified a set of miRNAs that are required to promote or limit the GC reaction through post-transcriptional gene expression regulation in B cells (**Figure 1** and **Table 1**).

miRNAs in the Regulation of Follicular Helper T Cells

The induction of the GC reaction is critically dependent on the colocalization of B cells with Tfh cells and interaction between the two. This GC B-Tfh cell interaction and the resulting intracellular signaling are also controlled by miRNAs expressed in Tfh cells. Remarkably, miR-146a downregulates the inducible costimulatory *Icos* expression in Tfh cells (34), and thus the expression of the two interacting molecules (ICOS and ICOSL) of this costimulatory pathway are negatively controlled in both cell subsets by the same miRNA. ICOS directly controls the migration of $CD4^+$ T cells from the T cell-B cell border into the B cell follicles of peripheral lymphoid organs (77). Importantly, ICOS signaling in T cells was shown to be important for miR-146a mediated Tfh and GC regulation (34). *Icos* expression is also negatively regulated by two other miRNAs whose expression is downregulated during Tfh differentiation, miR-101 and miR-103 (78, 79). Thus, ICOS co-stimulatory receptor expression is redundantly regulated by miRNAs in Tfh cells presumably to limit or end the GC reaction.

GC B-Tfh derived ICOS signaling is mediated by the PI3K/AKT pathway (80) and inhibited by PTEN phosphatase activity in Tfh cells (81). This key signaling pathway for Tfh activation and differentiation (3) is additionally regulated at different levels in Tfh cells by miRNAs from the miR-17-92 cluster. miR-17-92 cluster expression is induced early in T cell activation (48) and is repressed by BCL6, the critical transcriptional factor that regulates Tfh differentiation (82). T cell-specific miR-17-92 gain- and loss-of function mouse models showed that the microRNAs of the cluster are critical promoters of Tfh and GC B cell differentiation and antigen-specific antibody generation during both T-cell dependent immune responses and chronic viral infection (10, 48, 49). miR-17-92 cluster miRNAs regulate the ICOS-PI3K signaling pathway in Tfh cells through the simultaneous targeting of different pathway inhibitory components. miR-17-92 inhibits PTEN phosphatase expression upstream of AKT (10, 48, 51) as well as the downstream AKT phosphatase PHLPP2 (48). This pathway is additionally regulated in Tfh cells by Roquin, an RNA-binding protein that recognizes specific stem-loop structures in the 3'UTRs of target mRNAs and which interferes with miR-17-92 binding to an overlapping binding site in the *Pten* mRNA 3'UTR (83). Important miR-17-92 targets mediating other aspects of the Tfh differentiation program include the transcription factor RORα; responsible for the induction of IL-1R1 and CCR6 expression in Tfh cells (10), and CXCR5, a hallmark Tfh molecule that influences Tfh cell localization to follicles in which the ligand CXCL13 is expressed (82).

BCL6 represses the expression of a significant fraction of the miRNAs expressed in mouse $CD4^+$ T cells (82); however, the functional contribution of this repression to the Tfh cell transcriptional program has been characterized for few miRNAs outside the miR-17-92 cluster. BCL6 represses miR-31 expression in human Tfh cells through direct binding to its promoter (84). miR-31 inhibits the expression of CD40L, SAP, and BTLA, which are crucial for Tfh cell helper activity and cross-talk with B cells (85–87). Accordingly, Tfh cells forced to express miR-31 display decreased B-helper activity (84). Although BCL6 controls Tfh activity in humans and mice, the role of miR-31 is restricted to human Tfh cell differentiation, reflecting a species specificity on the action of some miRNAs.

Bcl6 gene expression is also regulated by miRNAs in $CD4^+$ T cells, and this regulation influences the generation of Tfh cells

TABLE 1 | Identified roles of miRNAs in the regulation of physiological GC responses and in GC-derived dysfunctions.

GC regulation miRNA	Role in GC physiology	Role in B cell neoplasia	Role in autoimmunity	Molecular mechanisms and targets
miR-17-92 polycistron (miR-17, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92a)	**Positive GC regulator**. Promotes GC responses, Tfh and GC B cell generation (10, 48, 49).	**OncomiR.** Promotes B cell GC-derived lymphoma (50)	**Promotes autoimmunity.** miR-17-92 expression in lymphocytes promotes spontaneous accumulation of Tfh and GC B cells, IgG anti-dsDNA antibodies and fatal immunopathology (48, 51).	Promotes proliferation and survival in lymphocytes by inhibiting the expression of *Pten* and *Bim* (51). Regulates differentiation and enhances ICOS-PI3K signaling by downregulating *Pten* and *Phlpp2* phosphatase gene expression in Tfh cells (10, 48, 49).
miR-155	**Positive GC regulator** Promotes GC responses and Tfh and GC B cell generation (12, 16, 52)	**OncomiR** Induces preB and mature B cell lymphomas (53–56)	**Promotes autoimmunity** miR-155 expression promotes autoimmunity in autoimmune mouse models of collagen-induced arthritis (57), systemic lupus erythematosus Fas^{lpr} (58, 59), and age-dependent miR-146a deficiency (52).	Regulates the GC reaction *via* B cell-intrinsic (12, 17, 18) and T cell-intrinsic mechanisms (52). Prevents LZ GC c-MYC^+ B cell apoptosis by downregulating *Jarid2* (19). Targets *Sfpi1* (17, 60) and *Aicda* (21, 22, 44) mRNAs and prevents P53 (27) and ERK activation through the inhibition of SHIP-1 (58) in B cells. Promotes *Prmd1*/BLIMP-1 expression and plasma cell differentation through PU.1-*Pax5* downregulation in B cells (20, 60). Regulates Tfh development and autoimmunity by modulating NF-κB, AP-1, and mTOR pathways (52) and promotes Tfh cellular proliferation and CD40L expression by repressing *Peli1* (61). Targets *S1pr1* in B cells from Fas^{lpr} lupus-like mice, and its expression is decreased in SLE patients (57, 59). Inhibits *Pu.1* in rheumatoid arthritis B cells (60). Promotes age-dependent inflammation associated to accumulation of Tfh, GC B cells and the generation of autoantibodies in miR146a deficient mice (62).
miR-217	**Positive GC regulator** Promotes GC B cell generation and GC responses (29).	**OncomiR** Overexpression in B cells leads to clonal GC-derived lymphomas (29).	NA	Downregulates DNA damage and repair response through *Nbs1*, *Xrcc2*, *Lig4*, and *Pds5b* gene expression downregulation and BCL6 stabilization (29).
miR-29	**Positive GC regulator** Promotes GC B cell generation after T-cell dependent immunization (63)	**OncomiR** Overexpression in B cells leads to B-cell chronic lymphocytic leukemia (B-CLL) development (64)	**Promotes autoimmunity** Promotes autoimmunity in collagen-induced arthritis (63)	Promotes B-cell proliferation (63, 64). Downregulates the expression of *Ddk6*, *Dnmt3a*, and the P53-responsive and tumor suppressor gene *Pxdn* (64).
miR-21	**Positive GC regulator** Promotes GC responses, Tfh and GC B cell generation (Schell SL J Immunol 2019, 202 (1 Supplement) 121.12; (Abstr) (65). Expression inhibited by BLIMP-1 during plasma cell differentiation (66).	**oncomiR** Induces B lymphomas dependent on continuous miR-21 expression (67).	**Promotes autoimmunity** miR-21 inhibition ameliorates disease in a lupus model (68)	Promotes B cell activation and proliferation. Activates the PI3K–AKT–mTOR pathway. Inhibits expression of *Pten*, *Pdcd4* (69), *Foxo* (70), *Fas* (65), and *Pdcd4* (68).
miR-28	**Negative GC regulator** Impairs CSR and memory B and plasma cell differentiation (33).	**Tumor suppressor** Efficiently inhibits tumor growth after intratumor or systemic administration of miR-28 synthetic mimics in various DLBCL and BL xenograft models and in a primary mouse BL (33).	NA	Inhibits BCR signaling and impairs B-cell proliferation and survival. Inhibits *MAD2L1, BAG1, RAP1B, p-AKT, p-ERK, NFKB2, IKKB* and *BCL2* gene expression (32, 33).

(Continued)

TABLE 1 | Continued

GC regulation miRNA	Role in GC physiology	Role in B cell neoplasia	Role in autoimmunity	Molecular mechanisms and targets
miR-146a	**Negative GC regulator** Limits Tfh and GC B cell generation and GC responses in T and B cells (34, 35, 52)	**Tumor suppressor** miR-146a knockout mice spontaneously develop B cell lymphomas and myeloid malignancies (71, 72). miR-146b and miR-146a knockout mice develop histologically distinct B cell lymphomas (73). miR-146a deficiency accelerates c-MYC-induced B cell lymphoma development (74).	**Inhibits autoimmunity** Loss of miR-146a in immune cells promotes autoimmunity (72) (52). Loss of miR-146a causes a B cell-intrinsic increase in anti- dsDNA auto-antibody production and spontaneous GC reactions (35).	Immunosuppressive roles in innate and adaptive immunity (72, 75). Downregulates the expression of signaling pathway components involved in GC B-Tfh cellular interactions, such as ICOSL-ICOS (34) and CD40-CD40L (35). Limits Tfh numbers by downregulating *Stat1* expression (75) and counterregulates miR-155 targets in Tfh cells, which is relevant to inhibit the generation of autoantibodies associated to age-dependent inflammation (52). Dysregulated overexpression promotes a lymphoproliferative syndrome and GC B cell expansion *via Fas* expression downregulation in GC B cells (76).

NA, not analyzed.

from T cell precursors. miR-10a, a miRNA highly expressed in mouse regulatory T cells (T_{reg}), has been proposed to attenuate the conversion of inducible T_{regs} to Tfh cells through *Bcl6* repression in mice (88). miR-346 has been suggested to repress *BCL6* gene expression in human Tfh cells (62).

Another key miRNA regulator of both CG B and Tfh cells is the positive GC regulator miR-155. Immunization of T cell-specific miR-155-deficient *Cd4*-Cre miR155$^{fl/fl}$ mice revealed impaired in GC B and Tfh cell generation and antigen-specific antibody production (52), revealing that miR-155 expression regulates Tfh development during immunization responses through T cell intrinsic mechanisms. The same study showed that miR-155 regulates different Tfh-cell targets important for Tfh development and autoimmunity in the NF-κB, AP-1 and mTOR pathways. Interestingly, miR-155 promotes Tfh cell accumulation during chronic, low-grade inflammation by counteracting the effect of miR-146a in Tfh cells (52). A later study showed that miR-155 promotes Tfh cell proliferation and CD40L expression by repressing expression of *Peli1*, a ubiquitin ligase that promotes the degradation of the NF-κB family transcription factor c-REL (61). These data suggest that miR-155 contributes to increased Tfh-mediated GC B activation through increased CD40L–CD40 interaction, which is known to be a limiting step in B cell clonal expansion, GC formation, isotype switching, affinity maturation, and the generation of long-lived plasma cells (89, 90).

Thus, miRNAs regulate Tfh cellular differentiation and interaction with B cells in the GC at multiple levels and through multilayer regulatory molecular circuits (**Figure 1**).

miRNAs IN GC-DERIVED B CELL NEOPLASIA AND AUTOIMMUNE DISEASES

Defects in GC regulation lead to immune diseases such as autoimmunity and mature B-cell neoplasia. These diseases are ultimately caused by the dysregulation of two distinct GC checkpoints; a breakdown of immune tolerance in autoimmunity and a surpassing of the DNA damage-tolerance threshold associated with Ig remodeling during CSR and SHM in B cell neoplasia. However, both diseases share a contribution from some of the mechanisms that promote GC dysfunction, including lymphoproliferative aberrant GC persistence, abnormal cellular components, and abnormal cellular signaling (91–93).

Recent studies addressing the contribution of miRNAs to these two GC-derived diseases revealed that dysregulated miRNA expression in GC B or Tfh cells can trigger B cell neoplasia or autoimmunity (**Figure 1**). Interestingly, several miRNAs that positively regulate the GC response also promote autoimmunity and B cell neoplasia (**Table 1**). For instance, miR-155, which promotes GC responses through T and B cell intrinsic mechanisms (12, 16, 52), also promotes autoimmune diseases characterized by switched auto-antibodies (52, 57–60, 94) and B cell neoplasia (53–56), likely through multilayer mechanisms that can lead to aberrant GC persistence due to increased

proliferation, reduced cell death and altered cellular signaling of Tfh and GC B cells (**Table 1**). Similarly, the miR-17-92 polycistron, which promotes GC responses by enhancing Tfh and B lymphocyte proliferation and survival by inhibiting the expression of *Pten* and *Bim* (10, 48, 49, 51), when overexpressed in different mouse models promotes the generation of spontaneous GCs, IgG anti-double–stranded DNA (dsDNA) autoantibodies linked to fatal immunopathology (48, 51), and B cell GC-derived lymphoma (50). Accordingly, miR-155 and miR-17-92 are upregulated in mature B cell neoplasia and GC-derived autoimmune diseases (95–97), suggesting their involvement in the enhancement of GC-derived human diseases. Other miRNAs that positively regulate GC responses and have been found to promote both autoimmunity and B-cell derived neoplasia include miR-29 and miR-21 (**Table 1**). Further studies are required to establish whether the switched autoantibodies generated in the context of positive GC miRNA regulator overexpression are derived from GC-derived plasma cells or are also generated from extrafollicular plasma cells.

Several miRNAs that negatively regulate the GC reaction have the opposite effect on B cell neoplasia and autoimmunity development, limiting the generation of GC-derived diseases (**Table 1**). miR-28 and miR-146a, well-characterized negative regulators of GC responses (33–35, 52), have both been found to exert tumor suppressor activity in B cell lymphoma development by limiting cell proliferation, promoting cell death and regulating cell signaling (33, 71, 73, 74) (**Table 1**). However, protection against autoimmune diseases has only been explored for miR-146a, which inhibits autoimmunity, anti-dsDNA autoantibody production (72), and spontaneous GC reactions (52) counterregulating miR-155 targets in Tfh cells (62) and through B cell-intrinsic mechanisms, likely by targeting CD40 signaling pathway components (35). Nevertheless, GC B cell miR-146a expression needs to be tightly regulated because forced overexpression promotes a lymphoproliferative syndrome *via Fas* downregulation (76). Thus, both super abundant or insufficient miR-146a expression are harmful for GC homeostasis.

Overall, these studies show that regulated miRNA expression is required to ensure proper GC responses and that GC-derived dysfunctions caused by miRNA alterations frequently lead to the development of both autoimmunity and B cell neoplasia through the disruption of post-transcriptional control mechanisms required for the maintenance of GC homeostasis, regulated cell signaling, cell death and proliferation. Further studies are needed to characterize with more detail the molecular mechanisms leading to both neoplastic transformation and autoimmunity caused by miRNA-dependent GC gene expression dysregulation.

CONCLUSIONS AND PERSPECTIVE

Studies by many groups in the field have shown that miRNAs play a key role in GC-response regulation and are required to prevent GC-derived autoimmunity and B cell neoplasia. The description of the role of dysregulated miRNAs in mature B cell oncogenic transformation and GC-derived autoimmunity has led to the clinical use of miRNAs as disease biomarkers with prognostic and predictive value and to the identification of targets for miRNA-based therapy (97, 98). The mechanisms leading to dysregulated miRNA expression in GC cells are poorly understood, and their characterization will likely provide new opportunities for therapeutic intervention. Strategies to restore or inhibit dysregulated miRNA expression have already established the therapeutic potential of miRNA modulation in *in vivo* models of GC-derived B cell neoplasia and autoimmunity (33, 44, 56, 68, 99–105). Moreover, synthetic miRNA mimics or anti-miR molecules are suitable for the generation of miRNA-based drugs that can be coupled to different types of nanocarriers and conjugates for effective delivery [reviewed in (106)].

The unique molecular features of miRNAs make them attractive tools for the development of miRNA-based therapies, and miRNA-based drugs are currently being tested in clinical trials for several diseases, including different types of cancer [reviewed in (97)]. This emerging and promising field faces a number of challenges regarding the clinical translation of miRNA-based therapies for B cell neoplasia and autoimmunity. Major challenges include i) the development of cell-type specific miRNA-based drug targeting approaches to improve specificity and reduce toxicity derived from miRNA delivery to healthy cells and ii) the development of models of human mature B cell neoplasia and GC-derived autoimmunity that faithfully recapitulate human disease to improve pre-clinical testing. The rapid pace of research in the field ensures the continuing excitement and expectations in building the path from basic science to translational miRNA-mediated GC regulation.

AUTHOR CONTRIBUTIONS

TF, IS and VY contributed substantially to the content and structure of this review. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: A European Patent Application titled 'miRNA compositions for the treatment of mature B cell neoplasms' EP16722679, EP17382740 was filed on March 3 2017.