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Regulation of B cell development and function by microRNAs

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Summary

microRNAs (miRNAs) have emerged as a new class of gene expression regulators whose functions influence a myriad of biological processes, from developmental decisions through immune responses and numerous pathologies, including cancer and autoimmunity. miRNAs are small RNA molecules that drive post-transcriptional negative regulation of gene expression by promoting the degradation or translational block of their target mRNAs. Here we review some of the data relating to the role of miRNAs in the regulation of the B cell lineage, with a special focus on results obtained *in vivo*. We start by giving a general overview of miRNA activity, including the issue of target specificity and the experimental approaches more widely used to analyze the function of these molecules. We then go on to discuss the function of miRNAs during B cell differentiation in the bone marrow and in the periphery as well as during the humoral immune response. Finally, we describe a few examples of the contribution of miRNAs, both as oncogenes and tumor suppressors, to the development of B cell neoplasias.

Keywords

microRNA; bone marrow; germinal center; Dicer; lymphomagenesis

General concepts on microRNA biology

Two decades ago, the Ambros' and Ruvkun's labs identified lin-4 as a small RNA that controls the larval development in the worm *Caenorhabditis elegans* through the negative regulation of lin-14 (1, 2). Strikingly, this RNA exerted its function not through the generation of a protein product but seemingly through pairing with complementary sequences in the 3'UTR of lin-14 mRNA. It was not until 2000 that a second small RNA, let-7, was shown to also control developmental transitions in *C. elegans* by the negative regulation of target mRNAs (3). Let-7 homologs were subsequently found in other organisms, including mammals (4), and soon a collection of several dozens of these small RNA molecules were cloned from worms, flies and mammals, and were collectively called microRNAs (miRNAs) (5-7). Today thousands of miRNAs have been identified in nearly 200 species (more than 1000 in humans alone) and they are recognized as a previously unforeseen regulatory layer of gene regulation critical to a plethora of biological processes. In mammals miRNAs are predicted to control the activity of $\approx 50\%$ of all protein-coding genes (8). Besides miRNAs, other classes of silencing small RNAs have been identified in animals, plants and fungi, including small interfering RNAs (siRNAs) and piwi-interacting RNAs (piRNAs), which will not be discussed here (reviewed in (9)).

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Several salient features of miRNAs distinguish them from classical regulators of gene expression, such as transcription factors and repressors. First, miRNAs do not encode a protein product, but instead they are biologically active as RNA molecules. Second, miRNAs are exclusively negative regulators of gene expression and act post-transcriptionally, either by promoting degradation of mRNA targets or by blocking their translation. This feature is thought to allow a fast and very precise regulatory response. Finally, miRNA activity on its target mRNAs typically results in a relatively mild (<2-fold) reduction in protein levels, which has led to the view that miRNAs act primarily as reinforcers of transcriptional programs conferring robustness to biological processes (10). However, the regulatory activity of miRNAs is in many respects similar to that of transcription factors and transcriptional repressors. Thus, like transcriptional regulators, a single miRNA can potentially regulate many targets to provide coordinated and simultaneous regulation of a network of genes in a particular tissue or at a specific developmental stage. Likewise, while target recognition by transcriptional regulators and miRNAs is based on nucleotide sequence specificity, in both cases, nucleotide sequence alone is insufficient to accurately predict functional targets. These similarities have important implications for the understanding of the physiological activity of miRNAs.

miRNA biogenesis, target specificity and regulation

miRNAs are 21-24-nucleotide long RNA molecules that are processed from longer RNA precursors (pri-miRNAs). Pri-miRNAs are either transcribed as independent genes or are included within intronic sequences of other genes. Pri-miRNAs fold into hairpins that are sequentially cleaved by two RNaseIII endonucleases, called Drosha and Dicer. Drosha cleavage generates a ~70-nucleotide long pre-miRNA that is exported to the cytoplasm, where Dicer further processes it into a 20-25 bp RNA duplex. One strand of this duplex is the mature miRNA, which is loaded onto the miRNA-induced silencing complex (RISC). The main components of the RISC complex are argonaute (AGO) proteins, which pair with the mature miRNA and guide it to its targets, and GW182 proteins, which act as downstream effectors for silencing. miRNA-RISC complexes bind to their target mRNAs and either induce their degradation or block their translation (the topic of miRNA biogenesis has been extensively discussed in excellent recent reviews (11-13).

The regulatory activity of miRNAs on target mRNAs is primarily determined by nucleotide sequence complementarity; however, the small size of miRNAs provides a relatively limited sequence for defining target specificity. In plants, miRNAs often pair with their targets through extensive complementarity. In contrast, in animals quasi-perfect alignment between miRNA and target mRNA is much more unusual, and typically the interaction involves the formation of partial duplexes that contain mismatches and nucleotide wobbles (14). The most important motif in a mature miRNA for determining target specificity resides in the 5' end, particularly in the stretch from nucleotides 2-7, called the seed. Accordingly, the 5' end is the most conserved portion of miRNA sequences in metazoans. It has been proposed that imperfect pairing at the 5' region can be compensated in some cases by interactions through the 3' end of the miRNA. Expectedly, these features are not stringent enough to faithfully predict miRNA targets on the basis of sequence complementarity alone. Numerous algorithms and predictive tools have been developed to aid in this task, which incorporate both sequence complementarity and evolutionary conservation criteria. These analyses estimate that a single miRNA can be expected to target several hundred mRNAs. However, the divergence between the predictions obtained with different algorithms makes the identification of miRNA functional targets one of the most challenging issues in the field (8, 15).

Gene silencing by miRNAs obviously entails that their expression be tightly regulated. Cellular miRNA levels are regulated by a variety of molecular mechanisms, including

transcription, processing and intercellular transport. Transcription of miRNA genes is regulated in a similar fashion to that of protein-coding genes and is frequently driven by RNAPolII. High-throughput approaches have shown that a significant fraction of miRNAs use their own transcription initiation regions, including miRNAs located in intergenic regions and others embedded within introns, and that transcriptional initiation can take place as far as 40 Kb from the pre-miRNA sequences (16, 17). However, there are relatively few detailed transcriptional analyses of individual miRNAs. Notably, myc and MYCN have been shown to regulate miRNAs with oncogenic and tumor suppressor potential, and p53 drives the expression of miRNAs that promote apoptosis and cell cycle arrest (18-21). In addition, various miRNAs have been shown to regulate their own expression through positive and negative feedback loops (reviewed in (11)). Although regulation of miRNA processing is far from being fully understood, it seems clear that the expression and activity of Drosha and Dicer endonucleases are critical and that these are affected by their dsRNA binding protein partners, DGCR8 and TAR. In turn, the exact position cleaved by Drosha and Dicer can affect not only the sequence identity of the mature miRNA but also its loading onto the RISC complex (reviewed in (11)). A mechanism of intercellular regulation by miRNAs has emerged that can constitute a critical additional layer to control their activity. It was first observed in plants and nematodes that RNA silencing signals can spread from the producing cells and travel from cell to cell and over long distances (reviewed in (22)). This concept has more recently been expanded to mammals, where miRNAs have been found contained in exosomes, secreted vesicles that can act as intercellular communicators (23, 24). Indeed, exosomal miRNAs regulate the expression of target genes in recipient cells during the immune synapsis, thus providing a trans-cellular mechanism of gene regulation (24). Notably, exosomes contain a specific repertoire of miRNAs that differs from that found in the cytosol, indicating the existence of a mechanism for selective sorting into these vesicles (23-25). The levels of mature miRNAs in a cell are thus influenced by the rate of transcription of the primary miRNA transcript, the efficiency of the processing of this transcript into mature miRNAs and the active transport of mature species from neighboring cells.

Approaches to the study of miRNA function

A number of experimental approaches are typically used to gain insights into miRNA function. Perhaps the most global strategy used to dissect the role of miRNAs in specific tissues or at defined developmental stages *in vivo* has been the analysis of mouse models in which the generation of mature miRNAs as a whole is prevented by blocking their biogenesis from precursors. This is the case of Dicer deficient mice. Germline deletion of Dicer leads to severe developmental defects and embryonic lethality (26), but conditional ablation of this gene has provided extensive information on the requirement of miRNAs for lymphocyte differentiation and function (27, 28). An obvious limitation of this approach is the difficulty of pinpointing the role of a specific miRNA in the observed phenotype by *in vivo* reconstitution with miRNA transgenes, since their maturation would also be blocked in the absence of Dicer. This issue can now be resolved by taking advantage of the identification of a miRNA, miR-451, whose expression levels are refractory to Dicer loss of function (29-31). The backbone of miR-451 can be used to reprogram the biogenesis of heterologous miRNAs, thus rendering them Dicer independent (31). This strategy will help the precise identification of individual miRNAs relevant to the phenotype conferred by Dicer-mediated global miRNA ablation.

One of the most popular approaches used to identify physiologically important miRNAs in biological processes is the systematic study of miRNA expression profiles. Initially these studies were performed by directional cloning and miRNA array analyses, but these strategies are being replaced by next generation sequencing and high-throughput qRT-PCR

(miRNA profiling techniques were recently reviewed in (32)). The underlying assumption of these analyses is that significant miRNA expression in a particular tissue or at a specific differentiation stage very likely reflects functional relevance. This assumption should, however, be taken cautiously in view of the recent estimate that over 60% of all miRNAs expressed in a particular cell type appear to have no silencing activity (33). In spite of this, it is reasonable to consider differential miRNA expression across sequential developmental or differentiation stages as a good indicator that those particular miRNAs play a role in the biological process under study.

miRNA function can be analyzed by gain- or loss- of function strategies, both *in vivo* and *in vitro*. Expression libraries have been used to identify miRNAs relevant to B cell function (34, 35), and a number of transgenic and knock-out mouse models have been generated to address the role of miRNAs in lymphocyte lineages (reviewed in (28, 36, 37)). In addition, miRNA loss-of-function can be achieved by the use of miRNA sponges—RNA molecules harboring repeated antisense miRNA sequences that can sequester miRNA molecules from their endogenous targets, acting as competitive inhibitors (38). Sponges have been successfully used to block the activity of miRNAs in the hematopoietic system *in vivo* after retroviral transduction and bone marrow transplantation (39, 40).

The ultimate challenge in the study of miRNA function is the identification of physiological targets. Predictive algorithms, such as miRanda, TargetScan or PicTar, are widely used for this task, although they pose the limitations described above. More recently, crosslinking immunoprecipitation (CLIP) of RISC components has been used in combination with high-throughput sequencing of RNA (HITS-CLIP) to identify miRNAs that physically interact with the silencing machinery and with potential target mRNAs *in vivo* (41, 42). However, this strategy does not provide definite proof that the identified interactions are functional. Very often, miRNA-mRNA target predictions or interactions have been validated in functional assays where putative target sequences are fused to a reporter gene, such as luciferase, whose expression is assessed in the presence or absence of the candidate miRNA. The limitation of this approach is that the context of the target sequence and the expression levels of both the reporter and the miRNA fail to reflect the natural cellular context in which the silencing takes place. To overcome this issue mice have been generated in which mutations are introduced into the 3'UTR of the putative mRNA target in order to disrupt miRNA binding *in vivo* (43, 44), thus allowing definitive validation of the silencing potential of a particular miRNA-mRNA target interaction. Notably, several studies have succeeded in pinpointing a particular mRNA target as the main contributor to the activity of a miRNA in *in vivo* complementation experiments, some of which will be discussed in detail below. However, it is important to keep in mind that, miRNAs resemble transcription factors in that they can operate as molecular switches through the simultaneous regulation of a large number of targets. Indeed, microarray analysis and quantitative proteomics approaches by stable isotope labeling with aminoacids (SILAC) have shown that individual miRNAs can promote the downregulation of hundreds of genes, a large fraction of which are potential direct targets (45-47). Therefore, a thorough search for miRNA targets should combine a prediction analysis (either *in silico* by the use of prediction algorithms or experimentally through HITS-CLIP) with high-throughput studies of the shift in gene expression induced by the miRNA (for instance by RNA-Seq or SILAC).

miRNA-mediated control of early B cell differentiation

Each individual B cell expresses a B cell receptor (BCR) for antigen with a unique specificity, so that the total pool of mature B cells is capable of producing antibodies that specifically recognize and respond to virtually any foreign antigen. This involves the generation of a hugely diverse repertoire of BCR specificities. The BCR is composed of two

identical immunoglobulin heavy (IgH) chains and two identical immunoglobulin light (IgL) chains. In all vertebrates, the genes encoding antibody variable regions are composed of groups of gene segments called V, D and J (for the IgH locus) or V and J (for the IgL locus). During B cell differentiation, a segment from each V, (D) and J group is assembled to give rise to a VDJ (or VJ) rearrangement; this combinatorial event is the basis of the primary antibody diversity. Assembly of antibody genes takes place through a process of site-specific recombination called V(D)J recombination and involves the introduction of DNA double strand breaks (DSBs) at specific recognition signal sequences flanking the V, D and J elements by recombination activating genes (RAG1 and RAG2), followed by repair. Developing B cells in the bone marrow (or fetal liver) first assemble their IgH locus in two successive steps, D_HJ_H and $V_HD_HJ_H$, at the so-called progenitor B (pro-B) stage. Pro-B cells that achieve a productive IgH rearrangement express an $Ig\mu$ chain together with the surrogate chains $\lambda 5$ and VpreB (preBCR). preBCR expression leads to a phase of vigorous clonal cell expansion of precursor B (pre-B) cells and the subsequent rearrangement of the IgL locus. As a result, a mature BCR is expressed for the first time in immature B cells, which are then ready to leave the bone marrow. Given the stochastic nature of V(D)J recombination, this developmental process is coupled to selective events that guarantee that only cells where a rearrangement has given rise to a functional preBCR and BCR receive the appropriate survival signals, while the rest undergo apoptosis. In addition, central and peripheral tolerance checkpoints ensure the elimination or inactivation of B cells expressing BCRs with self-reactive specificities (48) (Figure 1).

The first miRNA reported to have a role in B cell differentiation in the bone marrow was miR-181a (49). In this early study, miR-181a was identified as a miRNA differentially expressed in T and B cells and whose ectopic expression by retroviral transduction and bone marrow reconstitution promoted a substantial increase in the generation of B cells, both *in vitro* and *in vivo* (49). Interestingly, miR-181a was later shown to play an important role in the modulation of the thresholds of TCR signaling in thymocytes (50, 51), providing a nice example of a miRNA playing distinct roles in different cell lineages, presumably by affecting different sets of target genes. miR-181 is a family of miRNAs composed of the closely related miR-181a-d, which are expressed from three polycistronic transcripts (*miR-181a1b1*, *miR-181a2b2* and *miR-181cd*) where mature miR-181a and miR-181b can be generated from two independent clusters. In agreement with the results obtained in overexpression experiments (49), *miR-181ab1* deficient mice showed a mild decrease in the number of peripheral and germinal center (GC) B cells (52). Important questions remaining regarding the role of this interesting miRNA cluster in B cell differentiation include the identity of the precise developmental stage(s) affected in gain- and loss- of function approaches. It will also be important to assess the role of the *miR-181ab2* cluster in the B cell lineage and to identify its mRNA targets. In the following section we will discuss the function of miR-181b in GC B cells.

The global importance of miRNA regulatory mechanisms in the B cell lineage was directly assessed in mice in which Dicer was deleted at the earliest stage of B cell differentiation (Dicer^{fl/fl} mb1-Cre^{ki/+} mice) (53). miRNAs proved to be crucial, since Dicer deficient mice displayed a severe block of B cell differentiation at the pro-B to pre-B transition, at least partly due to massive apoptosis of Dicer-deficient pre-B cells, which resulted in an almost complete absence of B cells in the periphery. Combined bioinformatic analysis of miRNA and mRNA profiles showed that seeds for the miR-17~92 cluster were particularly enriched in the 3'UTRs of the mRNAs found to be upregulated in Dicer-deficient pro-B cells (53). The miR-17~92 cluster consists of 6 miRNAs that are processed from the same precursor transcript and was shown early on to have oncogenic potential (54), as discussed below. Indeed, miR-17~92 plays a major role in B cell differentiation, shown by the fact that germline deletion of this cluster also blocks the pro-B to pre-B checkpoint (55). Two of the

top predicted targets of miR-17~92 were the pro-apoptotic gene Bim and the tumor suppressor PTEN, both of which are strongly upregulated in Dicer-deficient animals (53). Accordingly, Bim expression was also upregulated in miR-17~92 -deficient mice (55) and both Bim and PTEN were downregulated in mice with ectopic overexpression of this cluster. Notably, the Dicer phenotype could be partially rescued by a Bcl2 transgene and by Bim deficiency (53), indicating a critical role of miR-17~92 in the regulation of Bim-induced apoptosis at this developmental stage (Figure 2).

The same pro-B to pre-B transition in B cell differentiation seems to be also regulated by another miRNA, miR-34a (40). The miR-34 family is transcriptionally regulated by p53 (19, 56, 57) and expression of these miRNAs is altered in various cancers (58), although their germline deletion in the mouse does not seem to influence cancer predisposition (59). Ectopic expression of miR-34a in bone marrow cells followed by reconstitution experiments promoted an increase in the proportion of pro-B cells and a concomitant decrease in pre-B cell numbers (40). Conversely, knock-down of miR-34a with a sponge construct increased the numbers of mature B cells. Target prediction combined with functional assays showed that Foxp1 is a *bona fide* target of miR-34a (40). Foxp1 is a transcription factor whose loss results in a severe blockade in B cell development (60). Indeed, a rescue vector containing a 3'UTR-less Foxp1 together with miR-34a was able to increase the number of pre-B cells, indicating that Foxp1 downregulation is a critical event in the developmental block induced by miR-34a (40). Although the downstream effectors of Foxp1 at this stage are not well characterized, this study identifies a p53-miR-34a-Foxp1 axis that may be critical for pre-B cell survival.

miR-150 has pleiotropic effects on B cell differentiation and function through the regulation of c-myb. c-myb is a transcription factor whose inactivation in the B cell lineage leads to several defects, including a partial block at the pro-B to pre-B transition and a decrease in conventional follicular cells and a subset of B cells, called B1 cells, which are responsible for the production of natural antibodies (61-63). miR-150 expression was detected in mature B and T cells (64, 65) and shown to target c-myb (66). Rajewsky and co-workers performed a comprehensive analysis of miR-150 function in the B cell lineage through germline deletion and ectopic expression approaches. They found that miR-150 deficiency leads to B1 cell expansion and an enhanced humoral immune response (see below), while ectopic expression of miR-150 blocks the pro-B to pre-B transition of bone marrow differentiation, most likely due to an increased rate of cell death (66). A similar developmental block was observed in a parallel study in which miR-150 was overexpressed *in vivo* by retroviral transduction of precursor cells followed by bone marrow transplantation (65). Importantly, although miR-150 overexpression only mildly reduced c-myb levels, this decrease was physiologically relevant, since mice with a heterozygous mutation in c-myb were impaired at the same pro-B to pre-B maturational stage (66). These data provide an example of how miRNAs can play key regulatory roles by keeping protein expression levels within the exquisite narrow range that guarantees optimal functionality.

miRNAs have also been shown to regulate the generation of B cells of fetal origin. The let-7 family of miRNAs act as regulators of stem-cell differentiation and have also been implicated in tumor suppression (67). Lin28 are small RNA-binding proteins (Lin28a and Lin28b) that regulate the activity of let-7 miRNAs by blocking their biogenesis (68). Lin28 is expressed in ES cells (69) and is a good predictor of cells that become induced pluripotent stem cells (iPSCs) (70). Indeed, ectopic expression of Lin28 (together with Sox2, Oct4 and Nanog) is able to reprogram human somatic cells to iPSCs (70). The immune system emerges in waves during development; the first of which arises during the fetal life and has unique properties, such as the efficient generation of innate-like lymphocytes, including B1 cells and $\gamma\delta$ T cells. A recent study showed that ectopic expression of Lin28b in adult bone

marrow hematopoietic progenitors can reprogram these cells toward fetal-like hematopoiesis, favoring the development of B1 and marginal zone B cells (as well as $\gamma\delta$ T cells) at the expense of B2, or conventional B cells (71). An additional layer of complexity was added to this regulatory network with the finding that Lin28a is negatively regulated by miR-125 (39). In turn, miR-125 overexpression in bone marrow precursors promotes myelopoiesis and compromises B cell differentiation by impairing the transition to the pre-B stage (39).

Generation of mature B cells involves a complex differentiation program that has long been known to be regulated by a number of transcription factors, including Pax5, PU.1, E2a and Ebf (72). The data summarized above show that miRNAs are also determining factors in the intricate network that regulates these events. Different miRNAs have been shown to act as positive (miR17-92) or negative (miR34a, miR-150, miR-125) regulators of B cell generation (Figure 1). Interestingly, alterations to the expression of these miRNAs in the bone marrow appear to principally affect the pro-B to pre-B transition, probably reflecting the importance of an optimal balance between proliferation and cell death at the developmental checkpoint governed by preBCR expression. In this regard, it is important to bear in mind that in the case of miR-34a, miR-150 and miR-125 this developmental block was observed in overexpression experiments, and that roles for these miRNAs at their endogenous levels at this particular stage are not completely established. This is a difficult issue to tackle, given the multiple effects some of these miRNAs have at different stages of B cell differentiation and function –and in some cases in other lineages as well. Another interesting consideration stemming from these reports concerns general regulatory mechanisms by miRNAs. In the case of miR-34a and miR-150, the two major identified targets are the transcription factors Foxp1 and c-myc, respectively. These findings reveal that although they are primarily post-transcriptional regulators, miRNAs can be involved in more complex loops of gene expression regulation, for instance by acting upstream of transcriptional regulators, possibly by promoting relatively subtle shifts in their expression levels.

miRNAs in peripheral B cell development and function

Terminal B cell differentiation takes place in the spleen and peripheral lymphoid tissues and serves to improve the competence and diversify the effector functions of B lymphocytes. Bone-marrow-derived immature B cells transit in the periphery to their final maturation through a number of transitional stages and give rise to either follicular (FO) or marginal zone (MZ) B cells (Figure 1). These subsets are functionally distinct: FO cells are mainly localized in B cell follicles and mount classical, T cell-dependent responses, while MZ B cells reside in the marginal zone areas of the spleen and can respond rapidly to blood-borne pathogens (73, 74). This maturational transition is associated with the peripheral B cell tolerance checkpoint that constrains the generation of lymphocytes expressing autoreactive BCRs (75). The FO versus MZ fate decision is functionally coupled to BCR signaling (74, 76) and it has been suggested that B cells bearing autoreactive BCRs are preferentially driven to an MZ fate (77, 78). When FO B cells encounter an antigen they can engage in the GC reaction, a complex biological process that generates long-lived memory B cells and plasma cells. At the molecular level this GC program involves two reactions that further diversify the Ig genes— somatic hypermutation (SHM) and class switch recombination (CSR), both of which are initiated by the activity of activation induced deaminase (AID). SHM introduces nucleotide changes in the V(D)J rearranged variable region of the IgH and IgL chains, allowing the production of B cells with a higher affinity for antigen. CSR is a region specific recombination reaction that replaces the primary μ constant region with a downstream constant region, giving rise to antibodies with different isotypes and therefore more specialized effector functions (reviewed in (79-82)). AID-mediated diversification of

the Ig genes entails the generation of DNA mutations or DSB intermediates. However, AID activity can also introduce mutations and generate DSB in other genes. Remarkably, these DSB intermediates have been shown to promote the generation of chromosomal translocations that juxtapose the Ig *loci* to heterologous genomic sites, including oncogenes, which are known to have lymphomagenic potential (83-87). This bystander effect of AID activity establishes a direct connection with the high propensity of germinal center B cells to undergo lymphomagenic transformation. Finally, B cells exit the GC as antibody-secreting plasma cells or long-lived memory B cells (Figure 1).

Work from several groups has helped to characterize the miRNA expression profiles of mature human and mouse B cell subsets. Two major studies analyzed miRNA expression profiles in B cells by small RNA library sequencing. Tuschl and co-workers sequenced 250 small RNA libraries from different organ systems, including 98 small RNA libraries from the human hematopoietic system. This work provided miRNA cloning frequency data from human total bone marrow, mature B lymphocytes and hematopoietic tumor biopsy samples, as well as from naïve and *in vitro* stimulated mouse spleen B lymphocytes. An important finding of this analysis is that only a third of the analyzed miRNAs were expressed with a high degree of tissue or cell type specificity (88). An independent study focused on identifying the human mature B cell miRNome and generated small RNA libraries from naïve, GC and memory human B cells and from a Burkitt lymphoma cell line. This study identified 178 miRNAs expressed in normal or transformed B cell libraries, including 75 previously unreported miRNAs. Interestingly, this work revealed that a large fraction of the most abundant miRNAs are shared by naïve and memory B cells. In contrast, GC B cells have a more distinct miRNA profile, suggesting specific functions of these miRNAs in this B cell type (89). More recently, Cassellas and co-workers combined miRNA-, mRNA- and ChIP-Seq next generation sequencing techniques to further characterize the miRNome during lymphopoiesis. In the context of B lymphocytes they analyzed miRNA expression of essentially all stages of mouse B cell ontogeny from pro-B cells to terminally-differentiated plasma cells, as well as all the mature human B cell subsets. In addition, their data showed that miRNA expression is tightly regulated by epigenetic, transcriptional and post-transcriptional mechanisms during mouse lymphopoiesis (90). Together, these studies provide an invaluable resource for singling out miRNAs that might play important roles in B differentiation and function. In this regard, it would be extremely useful to perform a bioinformatics analysis to carefully compare the sensitivity of these analyses and the conservation of miRNA expression in humans and mouse.

Two studies have used conditional Dicer ablation to address the effect of total miRNA depletion in mature B cells. To analyze the impact of miRNA depletion on terminal B cell differentiation our group generated a CD19-Cre^{Ki/+} Dicer^{fl/fl} mouse model (91). CD19-Cre promotes progressive deletion in the B cell lineage, which is completed at late stages of bone marrow differentiation (92). Dicer ablation in CD19-Cre^{Ki/+} Dicer^{fl/fl} mice did not significantly alter bone marrow differentiation, but resulted in a decrease in the generation of mature splenic and lymph node B cells. Peripheral B cell subsets were altered in CD19-Cre^{Ki/+} Dicer^{fl/fl} mice, with reduced numbers of conventional FO B cells and a relatively increased proportion of MZ B cells. To examine the miRNAs that might determine the FO versus MZ B cell fate, we compared miRNA expression profiles in control FO and MZ cells by microarray analysis. Strikingly, all differentially expressed miRNAs were expressed at higher amounts in FO B cells than in MZ B cells, suggesting that miRNAs are more determinant players in FO B cell differentiation. Bioinformatics analysis was conducted to determine whether genes with a known role in FO versus MZ cell generation were among the predicted targets of the differentially expressed miRNAs; this analysis predicted that miR-185, a miRNA overexpressed in FO B cells, would target Btk, a kinase that transduces signals downstream of the BCR. Btk has been shown to be involved in the generation or

recruitment of autoreactive B cells to the MZ area (78). We found that overexpression of miR-185 in primary B cells decreased Btk expression and that, conversely, Btk expression was increased in Dicer-deficient B cells. In the absence of Dicer BCR signaling was enhanced, as measured by the levels of Erk phosphorylation and the rate of CSR in *in vitro* stimulated B cells. In addition, Btk overexpression in wild type cells increased, whereas miR-185 expression decreased, CSR efficiency upon BCR stimulation. These results show that miR-185 inhibits BCR signaling through Btk and suggest that altered BCR signaling is responsible for the biased selection and commitment of CD19-Cre^{Ki/+} Dicer^{fl/fl} peripheral B cells. This notion was further reinforced by the finding that CD19-Cre^{Ki/+} Dicer^{fl/fl} peripheral cells express a skewed BCR repertoire, suggesting that the establishment of tolerance is incomplete in Dicer-deficient B cells. Importantly, Dicer deficiency promoted increased titers of autoantibodies in aged females, which led to immune complex depositions in kidney sections, lymphocyte infiltration and overall damage of glomerulus architecture, characteristic of autoimmune disease (91). In agreement with these findings, Hendriks and co-workers recently reported an autoimmune pathology in transgenic mice overexpressing Btk in B cells (93). These authors developed two transgenic mouse models for Btk overexpression in the B cell lineage that achieved a 3-7 fold increase of Btk levels. Their analysis revealed that Btk-overexpressing B cells are selectively hyperresponsive to BCR signaling and show enhanced Ca²⁺ influx, NF-κB activation, resistance to Fas-mediated apoptosis and defective elimination of self-reactive B cells. These mice, accordingly, develop a systemic lupus erythematosus (SLE)-like autoimmune disorder, characterized by autoantibody formation, kidney damage and perivascular lymphocyte infiltration. However, the autoimmune syndrome of these mice is not identical to that found in the CD19-Cre^{Ki/+} Dicer^{fl/fl} model, since Btk transgenic mice do not show gender-biased disease. This difference could be due to variations in the levels of Btk overexpression in B cells in the two models, to variations in strain susceptibility to develop autoimmune disease or to the contribution of other dysregulated genes in the Dicer deficient model. Moreover, in the collagen-induced arthritis and MRL-Fas(lpr) lupus models, treatment with a selective Btk inhibitor reduces autoantibody production and the development of autoimmune disease (94). Thus, different reports provide evidence that the regulation of Btk expression levels in B cells plays an important role in the development of autoimmune disorders. Together, our results have revealed a crucial role for miRNAs in terminal B cell differentiation and the establishment of B cell tolerance (Figure 2).

A second study developed Aicda-Cre^{Ki/+} Dicer^{fl/fl} mice to address the impact of global miRNA depletion on the differentiation of antigen-activated B cells in the context of GC responses (95). In this model Cre recombinase expression is under the physiological control of AID expression, so that Dicer is depleted specifically in antigen-activated B cells. This study showed that the absence of miRNAs severely compromises the GC response and that no memory B and plasma antibody secreting cells are generated in Aicda-Cre^{Ki/+} Dicer^{fl/fl} mice, establishing that miRNAs are essential for the B cell response to T dependent antigens. This is in part due to a defect in the proliferation of Dicer-deficient GC B cells, which expressed high levels of several negative regulators of genes involved in cell proliferation, including 8 cyclin-dependent kinases inhibitory (CDKi) genes. Importantly, the authors found that a high proportion of the miRNAs reported to be expressed at high levels in GC B cells (90) were predicted to target all 8 CDKi genes examined. The authors also showed that Dicer-deficient GC B cells have an increased apoptosis rate and expressed high levels of the pro-apoptotic molecule *Bim* (95). This study demonstrates that miRNAs are essential for antigen-dependent immunization responses and exemplify the complexity of the effects that miRNAs can induce in a particular cellular context (Figure 2).

The role of several individual miRNAs expressed in the B cell lineage has been studied using different genetic gain- and loss- of function approaches in *in vitro* and *in vivo* models.

One of the most extensively studied miRNAs in mature B cells is miR-155. miR-155 has emerged as a pivotal miRNA in the hematopoietic system and has a significant impact on the biology of multiple immune cells, including B and T lymphocytes, monocytes, dendritic cells and NK cells (96-101), and also plays an oncogenic role in some of these lineages (99, 102-104). miR-155 is expressed in mature B cells and is upregulated in GC B cells as well as in *in vitro* activated B cells (89, 90, 97, 105). Three independent studies showed that miR-155 is required for the GC response (96, 97, 106). miR-155 germ line deficient mice have an impaired humoral response to immunization, resulting in reduced numbers of GC B cells and reduced amounts of secreted switched antigen-specific antibodies (96, 97). In contrast, mice that conditionally overexpress miR-155 in mature B cells had increased numbers of GC B cells and showed high antigen-specific antibody secretion upon T-cell dependent immunization (97). Functional characterization of the cell subsets involved in the GC response showed that miR-155 was required for the proper function of DC, T and B cells (96, 97). *In vitro*, stimulation of miR155^{-/-} B cells resulted in reduced IgG1 production (96) and defective secretion of cytokines (TNF and LT- α) known to be critical for GC formation (97). Confirming these results, analysis of miR155^{-/-} mouse chimeras showed that *in vivo* B cells require miR-155 for the production of switched and high-affinity antibodies in a cell-autonomous manner (106). The gene expression profile of wild type and miR155^{-/-} B cells revealed that miR-155 regulates many genes in activated B cells (101 genes were significantly increased in miR-155-deficient B cells) (106), a significant proportion of which presumably are direct miR-155 targets (60 of them contain at least one seed match for miR-155 in their 3' UTR). Two of these putative miR-155 targets have been confirmed so far. Turner and co-workers showed that the transcription factor PU.1 is a direct target of miR-155 in activated B cells, and that its overexpression impairs CSR in *in vitro* stimulated wild type B cells (106). PU.1 had previously been implicated in early B cell differentiation (72) and in the signaling pathway downstream of the BCR (107). These results therefore add a new role for PU.1 in the B cell lineage, presumably as a negative regulator of plasma cell differentiation controlled by miR-155. Interestingly, the other characterized target of miR-155 in activated B cells is AID, the enzyme that initiates SHM and CSR. miR-155 is one of the top miRNAs predicted to target AID by algorithms such as TargetScan and miRanda. To assess whether AID is a direct target of miR-155 in B cells Nussenzweig and co-workers generated a knock-in mouse model in which the conserved miR-155 binding site in the 3' UTR of the AID gene was mutated (Aicda¹⁵⁵) (43). Aicda¹⁵⁵ B cells showed a modest increase in AID levels (2-3 fold higher than controls), demonstrating that AID is a direct target of miR-155 *in vivo*. Accordingly, Aicda¹⁵⁵ B cells showed increased CSR and c-myc/IgH translocation frequency in *in vitro* stimulation assays (43). Interestingly, the increase in the c-myc/IgH translocation frequency in Aicda¹⁵⁵ B cells (4-6 fold compared to control) was lower than in miR-155^{-/-} B cells (15 fold compared to control) (43), indicating that miR-155 targets additional mRNAs that cooperate to prevent AID-induced translocations in B cells. A similar mouse model was independently generated that harbored an AID BAC transgene with a mutated miR-155 binding site (44). The authors found that this mutation also resulted in higher AID expression levels *in vivo* and higher levels of CSR in *in vitro* stimulated splenic B cells (44). Additionally, the dysregulated expression of AID in these mice increased the mutation rate in an off-target gene for AID activity, and was associated with impaired affinity maturation (44). Together, these two studies provide the most physiological approaches used so far for the identification of a *bona fide* miRNA target in the B cell lineage. At the same time they reveal a functional paradox, in that although miR-155 acts globally as a positive regulator of several aspects of the GC reaction (96, 97, 106), it is at the same time a negative regulator of AID (43, 44), one of the key players in the same reaction (Figure 3). Recent data provide evidence that might help to reconcile these results. Bcl-6, a transcriptional repressor required for the GC reaction, has been found to repress the expression of miR-155 (Basso K. et al., *in press*). Although apparently counter-intuitive, it is possible that miR-155 and Bcl-6 are required at different

times during the GC reaction. Two functionally and anatomically distinct areas can be distinguished in GCs, called light (LZ) and dark (DZ) zones. The DZ is the main location for B cell clonal expansion and antigen receptor diversification, whereas the LZ is the site of antigen-driven selection. B cells perform iterative migratory cycles between the two zones to achieve affinity maturation (108). Bcl-6 might act as a positive regulator of AID expression and presumably other GC genes through miR-155 repression in the DZ, and conversely miR-155 expression in the LZ might regulate other aspects of the GC reaction, for instance by inhibiting PU.1. According to this speculation, miR-155 activity would therefore be spatiotemporally compartmentalized to provide various fine regulatory checkpoints during B cell activation.

AID expression is regulated by yet another miRNA, miR-181b (35). miR-181b was identified in a screen of 150 miRNA clones aimed at identifying miRNAs involved in the regulation of the GC reaction. Ectopic expression of miR-181b during B cell activation profoundly decreased the CSR rate. miR-181b expression levels are regulated concomitantly with B cell activation (35, 90). The CSR impairment induced by miR-181b was not caused by altered proliferation, survival or plasma cell differentiation. To identify putative miR-181b targets, we profiled mRNA expression shifts upon miR-181b overexpression. We found that 20% of the mRNAs expressed in B cells were downregulated in miR-181b expressing cells and that 60% of them contained miR-181b seed sequences in their 3'UTRs. Interestingly, AID was one of the most down-regulated of the mRNAs that contained miR-181b seed sequences. miR-181b overexpression reduced AID protein levels to approximately 50% of the levels found in control B cells. This reduction is physiologically relevant, since AID expression levels in activated B cell are limiting both for the CSR reaction and for the generation of c-myc/IgH chromosome translocations, as revealed in AID^{+/-} mice (109, 110). In turn, the reduction in AID levels promoted by miR-181b parallels the decreased efficiency in CSR. We further showed that the putative binding sequences in the AID 3'UTR can be directly targeted by miR-181b. Interestingly, AID and miR-181b have complementary expression profiles, with AID levels low in resting B cells and increasingly sharply in CSR-stimulated B cells, whereas miR-181b expression is highest in nonstimulated cells and drops upon stimulation. The same complementary expression pattern was found when miR-181b and AID expression levels were analyzed in a collection of Burkitt, mantle cell lymphoma and diffuse large B cell lymphoma (DLBCL) human B cell lines, supporting the idea that high expression of miR-181b prevents the accumulation of high levels of AID transcripts (35). Overall, these studies suggest that AID levels in B cells are controlled by different miRNAs with non-overlapping functions, so that while miR-181b would prevent premature AID activity in resting B cells and allow proper AID transcriptional activation upon stimulation, miR-155 would prevent excessive accumulation of AID transcripts in activated B cells (Figure 3).

Other miRNAs have been implicated in the regulation of different aspects of mature B cell biology, including the GC reaction and plasma B cell differentiation. miR-125b, a highly expressed miRNA in macrophages that participates in myeloid differentiation and myeloid leukemia (111, 112) is also specifically expressed in a subset of GC B cells (113, 114). miR-125b was shown to directly target BLIMP1 and IRF4, two transcription factors essential for post-GC plasma B cell differentiation (113-115). Accordingly, overexpression of miR-125b in B cells inhibited plasma B cell differentiation and Ig secretion *in vitro* (113). This miRNA could therefore enhance B cell diversification during the GC response by limiting their premature differentiation into plasma cells. Another miRNA implicated in mature B cell function is miR-150, in addition to its role during B cell differentiation. miR-150 expression is tightly regulated during B cell differentiation, being low in developing B cells, peaking in mature B cells and dropping upon B cell activation (66). Rajewsky and co-workers reported that miR150^{-/-} mice show increased secretion of

antigen-specific Igs in T cell dependent responses (66). Their data suggest that this may be due to enhanced expression of c-Myb in GC B cells. This is supported by the increased expression of the prosurvival protein Bcl2, a direct c-Myb target gene, and the reduction of cell death observed in activated mir150^{-/-} B cells upon IgM crosslinking (66). Additionally, we recently identified miR-217 as a novel positive regulator of GC reactions, finding that its expression is upregulated in activated and GC B cells (unpublished observations). To study miR-217 function in mature B cells *in vivo* we generated B cell specific gain- and loss- of function mouse models. miR-217 overexpression in B cells enhances T cell dependent immunization responses, improving the efficiency of GC formation, CSR and SHM, as well as the generation of plasma and terminally differentiated memory B cells. Conversely, when we inhibited endogenous miR-217 expression in B cells the generation of class-switched cells was diminished and the efficiency of SHM was lower than in control cells. These results show that miR-217 plays a physiological role in the secondary diversification of Ig genes in the context of the GC reaction (Figure 1).

In summary, several miRNAs have been identified that regulate terminal B cell differentiation and function, including antigen dependent and independent antibody repertoire diversification and the establishment of B cell tolerance. These regulatory schemes often involve mild changes in gene expression, which however result in profound functional alterations. In addition, expression profiling of miRNAs during B cell activation allow predicting that new players will emerge with critical roles in these events. Dissecting their functional interrelationships will undoubtedly be an exciting challenge.

miRNAs in B cell lymphomas

Disregulation of miRNA expression is a common event in cancer, including B cell lymphomas, and miRNA profiling is increasingly recognized as a valuable tool for cancer classification (116-120). Formal proof of the direct role of miRNAs in B cell lymphoma development came from the generation of mouse models in which alterations to miRNA expression levels had an impact on B cell lymphoma incidence. These studies revealed that miRNAs can function both as oncogenes (oncomiRs) and as tumor suppressor genes.

In the first study to demonstrate that miRNAs could promote lymphomagenesis, enforced expression of the miR-17~92 polycistron in hematopoietic stem cells from E μ -myc transgenic mice accelerated the generation of B cell lymphomas when these cells were transplanted into lethally irradiated recipient animals (54). In contrast, overexpression of miR-17~92 in wild type lymphocytes did not induce frank B cell lymphomas, although these mice died prematurely from lymphoproliferative disease and autoimmunity (121), indicating that the miR-17~92 cluster contributes to but it is not sufficient for lymphomagenesis *in vivo*. Lymphoproliferation and autoimmunity were due partly to repression of the tumor suppressor PTEN and the proapoptotic protein Bim, exerted directly by miR-17~92 in lymphocytes (53, 55, 121). When the tumorigenic potential of the individual miRNAs of the miR-17~92 polycistron were assessed in the E μ -myc B-cell lymphoma model, miR-19 emerged as the key proto-oncogenic component (122, 123). The proto-oncogenic activity of miR-19 was shown to be, at least in part, due to its promotion of cell survival through the repression of the tumor suppressor PTEN and the activation of the AKT mTOR pathway (122, 123).

In contrast to miR-17~92, overexpression of miR-155 in developing B cells is sufficient for neoplasia development (104). miR-155 expression is increased in various lymphoid malignancies, including chronic lymphocytic leukemia (CLL) and DLBCL (102, 124). The oncogenic potential of miR-155 was assessed in B cell specific E μ /V_H miR-155 transgenic mice, which develop transplantable acute lymphoblastic leukemia (ALL)/high grade

lymphoma in which SHIP and C/EBP β downregulation by miR-155 could be functionally relevant (104, 125, 126). miR-155 might promote B cell lymphomagenesis by targeting additional proteins, including HGAL—an inhibitor of lymphocyte cell motility (127) and SMAD5, whose downregulation renders lymphoma cells resistant to the growth-inhibitory effects of TGF β pathway (128). Other targets that might contribute to the proto-oncogenic potential of miR-155 include the mismatch repair proteins Msh2, Msh6 and Mlh1 (129), or other proliferation-related genes (130), which could endow miR-155 with mutator activity. miR-155 is thus a miRNA with lymphomagenic potential, although the exact mechanism by which it promotes transformation is far from being understood and seems to involve several different targets and pathways. It also remains unknown whether AID and PU.1, known to be targeted by miR-155, play a negative or positive role in the development of miR-155-induced lymphomagenesis or alternatively, if miR-155 regulates different targets at distinct developmental stages.

An interesting new concept in the oncomiR field arose from the work of Slack and collaborators, who showed that miR-21 contributes not only to the development of lymphoma, but also to tumor maintenance (131). miR-21 is overexpressed in most tumor types, including B cell malignancies (118, 132-134). A combination of Cre and Tet-off technologies was used to generate a mouse model that allowed conditional and inducible miR-21 overexpression in neural stem cells and the hematopoietic system (131). miR-21 overexpression in this mouse model promoted the generation of a transplantable pre-B cell lymphoma (131). Notably, switching off miR-21 expression in established tumors caused a rapid tumor regression mediated by a combination of apoptosis and proliferative arrest (131). These results demonstrate that miR-21 is a genuine oncogene and present the first case of “oncomiR addiction”, which may open new avenues for therapy.

Other studies have evidenced the proto-oncogenic role of various miRNAs in B lymphomas. miR-29, a miRNA whose expression is dysregulated in B-CLL, induces the generation of a CD5⁺ B-CLL-like disease when overexpressed in E μ -miR-29 transgenic mice (135). miR-125b, a GC miRNA that is overexpressed in B cell precursor-ALL and DLBCL (114, 136, 137), induces clonal B cell lymphomas in E μ -miR-125b transgenic mice (136). Finally, miR-217, a positive regulator of the GC reaction, promotes the generation of mature B cell lymphomas in a B cell-specific overexpression mouse model, establishing a functional link between the physiology of this developmental program and the molecular events leading to lymphomagenesis (our unpublished results).

miRNA profiling in lymphomas reveals that particular miRNAs are located in genomic regions that are frequently deleted in cancer (138-140). However, the first evidence that miRNAs could function as tumor suppressors in B cell lymphomas *in vivo* came from the work of Mendell and co-workers, who showed that c-myc-induced repression of a set of miRNAs contributed to lymphoma growth (20). These authors found that Myc induction in two models of B lymphoma resulted in widespread downregulation of miRNA expression, presumably via direct binding to their promoters (20). Importantly, re-expression of a set of c-myc repressed miRNAs including mi-R34a, miR-150, miR-195/497 and miR-15a/16-1, abrogated the tumorigenic potential of B cell lymphoma cells in SCID mice (20). Conditional knockout models have been used to directly assess the contribution to B lymphomagenesis of the minimal deleted region (MDR) within 13q14, which is deleted in more than 50% of B-CLL (141). MDR-13q14 encodes the DLEU2, a long non-coding RNA, and the miR15a/16 cluster (5, 138). Interestingly, mice in which only the miRNA cluster was deleted showed a clonal pre-leukemic expansion of CD5⁺ B cells, whereas mice in which the MDR was deleted developed a more aggressive CLL-like disease (141). This study further showed that the miR15a/16-1 cluster limits the proliferation of B cells by targeting multiple genes involved in the G₀/G₁-S phase transition (141). However, the more

aggressive phenotype of MDR-deleted mice suggests that additional genetic elements within the MDR locus contribute to the tumor suppressive function. Another study revealed a role for a set of p53-induced miRNAs in a multiple myeloma (MM), a neoplasm of plasma B cells (142). The tumor suppressor p53 promotes the expression of miR-192, miR-194 and miR-215, which in turn directly target MDM2, a negative regulator of p53 (142). This regulatory loop could be valuable, since re-expression of these miRNAs in MM cell lines increased the therapeutic action of a MDM2 inhibitor *in vitro* and in a mouse xenograft model (142). In addition, this set of miRNAs impaired the migration and invasion of MM cell lines in NOD-SCID mice through the inhibition of IGF-1 and IGF1-R (142). Finally, miR-146a has been shown to function as a tumor suppressor miRNA for myeloid and B cells *in vivo* (143). miR-146a is expressed in various immune cell types and its expression is deregulated in different cancers, including DLBCL and CLL B cell lymphomas (114, 144, 145). Germ-line miR-146a deficient mice develop a chronic inflammatory phenotype that progresses to splenic myeloid sarcoma and, less frequently, into B cell lymphomas (143). The authors went on to show that this effect of miR-146a is mostly due to negative regulation of NF- κ B by miR-146a, with deletion of the NF- κ B p50 subunit effectively rescuing the myeloproliferative disease (143). These data provide a good example of a link between miRNA regulatory activity, inflammation and cancer.

Overall, these studies open a new perspective to the understanding of the molecular mechanisms underlying oncogenic transformation, in which miRNAs can play a fundamental, previously unforeseen role.

Concluding remarks

The last few years have seen an outburst of research into the regulation of diverse biological processes by miRNAs. Within the B cell lineage, this effort has led to the identification of a number of miRNAs that impinge on critical differentiation or activation checkpoints, including miR-181, miR-17~92, miR-34, miR-150 and miR-155. A number of miRNAs are, moreover, now known to contribute to B cell lymphoma development, either as oncogenes or as tumor suppressors. A considerable effort has been made in this and other fields to pinpoint individual mRNA targets that could account for the functional impact of a miRNA. However, it is already evident that miRNAs very often play pleiotropic roles in intertwined regulatory networks, and that high-throughput approaches seem to be required to identify more complete sets of target genes at defined developmental stages. Continuing implementation of proteomics approaches, gene expression analysis by next generation sequencing and HITS-CLIP techniques will undoubtedly prove invaluable. Importantly, miRNAs are emerging as a new class of molecules with clinical potential. Their value as diagnostic and even prognostic markers is already a reality in cancer and other pathologies. Moreover, the possibility of manipulating the expression of miRNAs *in vivo* by the delivery of miRNAs or miRNA inhibitors has opened new and exciting therapeutic perspectives for the treatment of numerous diseases. We await new developments in this rapidly expanding field with anticipation.

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Figure 1. miRNAs play crucial roles in B cell development

Scheme depicting the developmental stages of B cell differentiation in which particular miRNAs have been shown to play a role.

Figure 2. Stage-specific deletion of Dicer reveals a key role of miRNAs at different B cell developmental stages

The role of Dicer ablation in the B cell lineage was assessed by Cre-mediated conditional deletion at three different developmental stages with mb1-Cre (grey), CD19-Cre (blue) and Aicda-Cre (orange) deleter strains. Bars on the left show the kinetics of Dicer deletion. The most prominent functional effect observed in each model is summarized in the middle column. The cartoon on the right depicts the specific developmental blocks with shaded arrows and boxes and the main miRNAs and mRNA targets involved.

Figure 3. Roles of miR-181b and miR-155 in mature B cells

miR-181b and miR-155 target AID, an essential enzyme for CSR and SHM in GC B cells that can also promote the generation of oncogenic chromosomal c-myc/IgH translocations. Moreover, miR-155 inhibits the generation of c-myc/IgH translocations by targeting other, not yet identified, genes. miR-155 is also required for the GC response and promotes the generation of class-switched cells by targeting the transcription factor PU.1. In addition, miR-155 overexpression in B cells has been shown to induce B cell lymphomagenesis.