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MicroRNAs and B cell receptor signaling in chronic lymphocytic leukemia

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Abstract

The relative expression levels of certain microRNAs (miRNAs) correlate with known prognostic markers in chronic lymphocytic leukemia (CLL), such as leukemia-cell expression of zeta-associated protein of 70 kDa (ZAP-70), use of unmutated immunoglobulin heavy-chain variable region genes (IGHV), chromosomal abnormalities or dysfunctional p53. Here we review studies that provide evidence suggesting that certain miRNAs (e.g. miR-155, miR-17-92, miR-181, miR-29) can regulate the activated phenotype of CLL cells and/or fitness of the surface-immunoglobulin (sIg) B cell receptor (BCR) complex expressed by CLL cells, thereby accounting for the differential leukemia-cell expression of these miRNAs in different CLL prognostic subgroups. How these miRNAs influence cellular activation and/or BCR signaling through the post-transcriptional regulation of critical signaling molecules (e.g. Lyn, Syk, BTK, SHIP-1, SHP1) is a topic of current research.

Keywords

CLL; miRNA; ZAP-70; BCR-signalling; prognostic marker; immunoglobulin

Introduction

The seminal observation that the miR-15a-16-1 cluster located at the 13q14 region is deleted or deregulated in the leukemia cells of ~ 50% of all cases of chronic lymphocytic leukemia (CLL) [1] provided the first evidence that the small non-coding RNAs, known as microRNAs, could contribute to human disease. We now know that there are several hundred functional microRNA genes in the human genome (miRBase v. 19) that can each influence post-transcriptional expression of multiple genes (dozens to hundreds) by inhibiting the translation and/or stability of target messenger RNA (mRNA) [2]. Since the initial observation that miR-15a/16-1 might contribute to CLL pathogenesis, there have been numerous studies examining the potential contribution of these and other microRNAs to the

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pathogenesis and/or progression of CLL. We reviewed this rapidly accumulating knowledge in this journal in 2009 [3], when the functional consequences of such differences in microRNA expression were largely unclear. This brief review focuses attention on selected microRNAs that are frequently studied in CLL and that appear most likely to contribute to the (dys)regulation of BCR signaling in CLL B cells (Table I).

Role of microRNAs in BCR structure and signaling

The stage-specific expression of various microRNAs during the maturation of B cells suggests that they play a role in normal B cell development [4]. Consistent with this notion are studies in mice made deficient of the enzyme Dicer, which is required for the production of mature microRNAs. Mice with conditionally deleted Dicer in CD19+ cells develop self-reactive antibodies and autoimmunity associated with increased numbers of marginal-zone B cells and decreased-numbers of follicular B cells [5]. The molecular basis of these observations remains largely unclear, but might relate to recent studies that indicate that B cell receptor (BCR) signaling and immunoglobulin production can be regulated by microRNAs [2,6]. In turn, BCR stimulation can alter the expression of certain microRNAs, and such BCR-regulated microRNAs might contribute to the orchestration of B cell proliferation, apoptosis and/or sensitivity to BCR signaling [6].

Analysis of the differential expression of microRNAs by CLL cells that use unmutated versus mutated immunoglobulin heavy-chain variable subgenes (IGHV) and/or express versus lack expression of the zeta-associated protein of 70 kDa (ZAP-70) revealed that microRNAs have specific expression profiles in these CLL subtypes, and are also different from normal B cells. Altogether ~ 18 microRNAs were identified as differentially expressed between CLL cases divided based on the germline homology of their IGHV and/ or ZAP-70 expression (reviewed in [3]). Some of these differentially expressed microRNAs (such as miR-29, miR-181) were implicated in the regulation of anti-apoptotic proteins myeloid leukemia cell differentiation protein 1 (Mcl1) or T-cell leukemia 1 (Tcl1) [3], but an unbiased approach for identification of genes regulated by these microRNAs is still lacking. Results of these studies, however, suggested that microRNAs might contribute to regulation of the activated phenotype of CLL B cells and/or heterogeneity in BCR signaling propensity observed among CLL cell samples. Following this hypothesis, two recent publications compared the changes in microRNA expression after stimulation of BCR or Toll-like receptors on normal and CLL B cells. Li and colleagues [7] noted that the microRNA expression profile of CLL cells resembles that of B cells activated by treatment with anti-immunoglobulin M (IgM) or CpG. In general, the differences in microRNA expression profile between “ germinal center ” and “ non-germinal center ” B cells resemble those observed by expression profiling of protein-coding genes [4]. Additionally, Bomben and colleagues [8] suggested that the microRNAs induced upon treatment with CpG regulated genes encoding pro-survival and growth-promoting proteins induced by stimulation of Toll-like receptors. This appears in part to be regulated by c-MYC, which can bind the promoters of microRNAs, such as those of the miR-17-92 cluster, to induce expression of such microRNAs. Additionally, certain Toll-like receptors can also be directly stimulated upon binding microRNA molecules secreted by different cell types and/or transported via exosomes. This could be an important link between the tumor microenvironment and the

intracellular machinery that orchestrates B cell activation and responsiveness to microenvironmental stimuli.

It is noteworthy that many of the microRNAs that are affected by BCR-stimulation in CLL and/or normal B cells are also those that are differentially expressed between cases of CLL with favorable versus unfavorable prognosis (Table I). This suggests that microRNAs might directly or indirectly influence activation in response to BCR ligation and/or disease progression in CLL. However, only a few of the micro-RNAs are known to behave like classical proto-oncogenes, with the possible exception of miR-155, which is expressed at high levels in B cell malignancies and can induce polyclonal B cell expansion and lymphomas in transgenic mice [2]. MicroRNA-155 is up-regulated in response to BCR ligation, and appears to play a role in the development of T and B cell immunity, particularly the formation and maintenance of plasma cells secreting IgG [9]. Microarray analysis revealed that deletion of miR-155 may result in the enhanced expression of a large number of target genes [2], most notably the transcription factor PU.1, activation-induced cytidine deaminase (AID) and BCR-associated phosphatase SHIP-1. As is likely the case with all microRNAs, however, the effects of increased or decreased expression of miR-155 are context-dependent and vary depending upon the relative expression of numerous target mRNAs in any given cell type. Recently, we found in CLL cells that PU.1 is likely targeted by miR-155, which is expressed depending upon the methylation status of its promoter and the transcription factor MYB [10]. The induced expression of miR-155 in B cells following BCR ligation requires cellular activation via extracellular signal-regulated kinase (ERK)-, Jun N-terminal kinase (JNK)- and nuclear factor-κB (NF-κB)-dependent pathways, which are themselves regulated by microRNAs [11,12]. In this regard, it is noteworthy that Epstein – Barr virus (EBV)-encoded latent membrane protein-1 (LMP1) contributes to EBV ’ s oncogenic potential by up-regulating miR-155 in the infected B cells through activation of NF-κB [12].

It is not surprising that the studies of miR-155 provided the first functional evidence for the role of microRNAs in BCR signaling in CLL. It was found that CLL cells with low levels of the miR-155 target SHIP-1 tend to have higher expression of miR-155 and higher capacity for BCR signaling ([13] and manuscript in preparation). In CLL it seems that miR-155-mediated regulation of SHIP-1 phosphatase plays an important role in counterbalancing (inhibiting) the BCR signaling capacity. However, the role for other microRNAs in regulation of the complex BCR signaling cascade and BCR-engaged kinases (such as Lyn, Syk, BTK) remains one of the highly interesting, yet poorly explored, questions in the biology of normal and malignant B cells.

The aberrant expression of miR-155 was observed not only in lymphoproliferative disease, but also in many autoimmune conditions, including rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus, which makes it a potentially interesting therapeutic target. In recently published studies, investigators used anti-sense oligonucleotides specific for miR-155 to inhibit the growth of B cell lymphomas in mice [14]. These studies demonstrated the feasibility of using anti-miR oligonucleotides to inhibit tumor growth *in vivo*.

That miR-155 is expressed at relatively high levels in aggressive CLL stands in contrast to what is observed with many other microRNAs in this disease, in which relatively low levels of certain microRNAs are associated with more aggressive disease (Table I). For example, activation via BCR ligation can reduce the levels of miR-29c, miR-150, miR-181b or miR-223, and relatively low-level expression of these microRNAs is found in CLL cells of patients with shorter overall survival and/or time to initial treatment [7,15 – 17]. Reduced expression of miR-181b, which is associated with increased expression of its notable target genes (e.g. Mcl1 and Bcl2), is a marker of disease progression in CLL [18]. The down-regulation of putative tumor-suppressor microRNAs in aggressive cancer subtypes is a frequent phenomenon, and in the case of CLL may lead to overexpression of anti-apoptotic molecules or potentiate BCR signaling. We have found that a large-scale microarray-based approach to the identification of target mRNAs regulated by such down-regulated microRNAs is feasible in CLL, and could help to better uncover the extent of their contribution to BCR pathway (dys)regulation ([17] and manuscript in preparation). The integration of microRNA and gene-expression profiling represents a novel approach that can also be used to identify protein-coding genes with important functions in CLL [17].

It remains unclear to what extent the deregulation of microRNAs represents the cause or the consequence of aberrant BCR signaling or if it contributes, for example, to the maintenance of unbalanced BCR activation in CLL cells, thus favoring malignant B cell clone survival. It is evident that microRNA expression in CLL is influenced by aberrant methylation and acetylation of their promoter regions, which could be due to the response to BCR stimulation as shown previously for protein-coding genes. In these situations, the use of histone deacetylases can induce/restore microRNA expression and sensitize CLL cells to apoptotic stimuli [19], which partially uncovers the mechanism of action for chromatin structure-modifying drugs.

Considering the importance of immunoglobulin gene rearrangement in the development of normal and neoplastic B cells, it is interesting to note that the human Ig locus encodes a microRNA, namely miR-650, which is housed in several light chain variable subgenes of the V2 family. The regulation of miR-650 appears to be coupled with expression of the immunoglobulin lambda light chains, which represents a peculiar mechanism for the regulation of microRNA levels. The expression of miR-650 is associated with CLL prognosis and influences B cell proliferation through regulation of several target genes [20]. Currently, it remains unclear whether any other immunoglobulin genes give rise to non-coding RNAs and how this developed in the evolution of the immunoglobulin locus.

Conclusions

Recently published studies provide evidence that microRNAs are involved in the BCR pathway and could contribute to the (dys)regulation of BCR signaling in malignant CLL B cells. MicroRNAs frequently associated with CLL biology and the effect of B cell activation/BCR stimulation on their expression are summarized in Table I. These novel observations at least partially explain the differences in microRNA expression between different CLL prognostic subtypes. Conceivably, microRNA inhibitors or mimics could be

used to manipulate the response to BCR signaling, a prospect that has potential therapeutic implications for patients with B cell malignancies.

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Table I

MicroRNAs frequently associated with CLL biology and the effect of anti-IgM/CpG stimulation on their expression in B cells/CLL cells.

MicroRNA	Chromosomal location	Expression in CLL*	Target gene(s)†	MicroRNA expression after stimulation of:		
				Normal B cells with anti-IgM [7]	Normal B cells with CpG [7]	CLL cells with CpG [8]
miR-155	21q21	Higher expression in cells with unmutated IGHV, ZAP-70 +, 17p13 deletion	PU.1, AID, SHIP, C/EBPβ	↑	↑	↑
miR-150	19q13	Lower expression in cells with unmutated IGHV or ZAP-70 +	MYB	↓	↑	↓
miR-181b	Homologous miR-181b-1 (1q32) and miR-181b-2 (9q33)	Down-regulated with disease progression; lower expression in cells with 17p13 deletion	MCL1, TCL1, BCL2	↓	↓	—
miR-29a/b	7q32	Lower expression in cells with unmutated IGHV, ZAP-70 +,	CDK6, TCL1, MCL1	↑ (miR-29a)	↑ (miR-29a)	— (miR-29a)
miR-29c	1q32	17p13 deletion and/or p53 mutation; expression of miR-29b regulated by histone acetylation		↓ (miR-29b/c)	↑ (miR-29b/c)	— (miR-29b/c)
miR-223	Xq12	Lower expression in cells with unmutated IGHV or ZAP-70 +	ND	↓	↓	—
miR-34a	1p36	Lower expression in cells with 17p13 deletion and/or p53 mutation	BCL2, SIRT1	ND	↑	—
miR-17-5p	13q31	Lower expression in cells with 17p13 deletion and/or p53 mutation	E2F1, CDKN1A, P21	ND	—	↑
miR-21	17q23	Higher expression in cells with17p13 deletion	BCL2	ND	—	—
miR-106b	7q22	miR-106b is induced in response to histone deacetylase inhibitors, which facilitates p53-independent apoptosis in CLL cells	ITCH	ND	—	—
miR-650	22q11	High expression in cells utilizing V2 family for lambda light chain immunoglobulin variable subgene	EBF3, ING4, CDK1	ND	ND	—
miR-15a, miR-16-1	13q14 (homologous miR-16-2 at 3q25)	Lower expression in cells with deletion 13q14; higher expression in cells with unmutated IGHV or ZAP-70 +; expression regulated by histone acetylation	CCND1, BCL2, P53	ND	—	—

CLL, chronic lymphocytic leukemia; IgM, immunoglobulin M; ↑, up-regulation; ↓, down-regulation; —, no change in expression; ND, not determined.

*For references see text.

†Target gene validated in CLL or related B cell malignancy.