The diagnosis, prognosis, and therapeutic application of MicroRNAs in haematological malignancies

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Objective: MicroRNAs (miRNAs) are small noncoding RNA molecules that participate in vital cell processes such as proliferation, apoptosis, and differentiation. In recent years, they have been proven to play vital roles in haematological malignancies. In this review we briefly introduce some basic knowledge of microRNAs and summarize their ectopic expression in haematological malignancies, especially in leukaemia. We will also discuss the potential of microRNAs in the diagnosis of leukaemia, in the determination of the clinical prognosis of diverse subtypes, and in targeted therapy.

Discussion: Despite current adoption of novel biological agents combining traditional chemotherapy regimens, leukaemia remains to have undesirable clinical outcomes due to inaccurate diagnosis, invasiveness of the disease, and patients’ intolerance to chemotherapy, thus brand new therapeutic directions are urgently needed. MiRNAs regulate gene expression by means of binding to the 3'-untranslated regions of corresponding mRNAs, leading to the degradation of targeted mRNA or the inhibition of translation. It has been confirmed that they can either function as tumour inhibitors, or may trigger tumourigenesis in certain situations, this specific dual characteristic undoubtedly attract scientists to explore their roles in haematological malignancies. It is of great necessity to summarize the roles of miRNAs in haematological malignancies diagnosis, prognosis evaluation, and clinical treatment.

Conclusions: Future studies may take full advantage of miRNAs detection in diagnosing, in choosing targeted biological therapy, and in avoiding predictable side effect, thus the overall survival rate and cure efficiency of leukaemia should improve.

Keywords: MicroRNAs, Haematological malignancies, Ectopic expression, Biomarkers, Clinical prognosis, Targeted therapy

Introduction

MicroRNAs (miRNAs) are a class of small, noncoding RNA molecules of 18–25 nucleotides in length that regulate approximately 30% of all protein-coding RNAs. They regulate gene expression by the induction of mRNA degradation or the inhibition of translation by binding to the 3'-untranslated regions of target mRNAs. MiRNAs take part in various biologic processes that involve development, proliferation, apoptosis, and differentiation and have been proven to act as both oncogenes and tumour suppressors. MicroRNA biogenesis begins in the nucleus, and genomic sequences that code for miRNAs are transcribed by RNA polymerase II to form primary miRNA transcripts (pri-miRNAs). Pri-miRNAs are further cleaved within the nucleus by means of the ‘microprocessor complex’, which consists of Drosha, an RNA processing enzyme, and its co-factor DiGeorge syndrome critical region gene 8, to form a 60- to 110-nucleotide hairpin structure (pre-microRNA). The pre-miRNAs are then actively transported to the cytoplasm by Exportin 5 and are cleaved by DICER1 (Dicer) into mature miRNAs before being loaded into the RNA-induced silencing complex.

Upon receipt of the proper signals, haematopoietic stem cells can rapidly differentiate into blood cells of distinct lineages while maintaining their pluripotency. The entire process is tightly controlled by a complex network of stimuli from inner and outer spaces, transcription factors, cytokines, signalling pathways and many other important biological molecules. It has been shown that miRNAs specifically target many of these factors and thus play vital roles in the process of haematopoiesis.
Hundreds of miRNAs have been proven to be involved in the process of stem cell self-renewal and differentiation, and their expression may differ greatly in various lineages. It was observed in one study that miR-17, -24, -146, -155, -128, and -181 can block the early differentiation of hematopoietic cells into more mature forms, and miR-16, -103, and -107 may block the differentiation of later progenitor cells, whereas miR-221, -222, and -223 control the terminal stages of hematopoietic differentiation. Their abnormal expression obviously leads to the accumulation of immature progenitor cells, which may be one of the mechanisms of tumorigenesis. The members of the miR-125 family have been shown in several groups and are over-expressed in hematopoietic stem and progenitor cells, and their expression decreases upon differentiation. Several recently discovered miRNAs are lineage specific. For example, GATA-1 activates the transcription of miR-451 and leads to the down-regulation of c-Myc, which is a transcription factor and oncogene that is involved in the self-renewal of hematopoietic stem cells. As a result, the progenitor cells differentiate into the erythroid lineage. In the lymphoid lineage, as an important regulator, the deletion of the miR-17-92 cluster leads to an increase in the expression of the pro-apoptotic protein Bim in B cells, which prevents pro- to pre-B cell development.

Over the years, various methods have been used to detect ectopic expression in human diseases, including haematological malignancies. Links between genomic localizations of miRNAs and cancer-associated genomic-regions (CAGRs) enlightening scientists to discover their involvement in cancers for the first time. Joshi D pointed out that a high proportion of miRNA genes are encoded in the CAGRs, fragile sites, and regions concerning loss of heterozygosity and amplification. The miRNA signature can be used in the diagnosis of diseases and can even be used to distinguish between myeloid and lymphoid tumour lineages. They can also function as prognostic indicators of haematological disease and can be used to differentiate high-risk, immediate-risk, and low-risk cancers. Their specific dual characteristics as both an oncogene and a tumour suppressor has led scientists to create tools for future therapeutic intervention, such as antagonists or miRNA restoration techniques. This review mainly introduces the ectopic expression of miRNAs in leukaemia and their role in diagnosis, prognosis, evaluation, and targeted therapy.

The role of miRNAs as novel biomarkers in the diagnosis of leukaemia

Many patients do not have symptoms during the early stages of disease, it is therefore of great necessity to identify predictive biomarkers to detect their changes in the early stages of disease and to take forceful action. Numerous studies have investigated the potential value of circulating miRNAs for the detection of leukaemia.

(1) miRNAs as biomarkers in acute myeloid leukaemia (AML)

AML includes a heterogeneous group of neoplastic hematopoietic diseases that are characterized by the differentiation obstacle of myeloid cells at early stages, and the accumulation of immature hematopoietic stem and progenitor cells leads to diverse clinical symptoms. The most remarkable traits of AML are the accompanying specific cytogenetic translocations and molecular abnormalities. It has been demonstrated that microRNA signatures are associated with these genetic mutations.

Four major rearrangements in AML are the t (8;21), inv (16), t (15;17), and MLL/11q23 translocations, which account for about 30% of all AML cases, the World Health Organization has adopted them as the criteria for subclassification of AML. In a cohort of 100 primary AMLs, those with the t(15;17) translocation were validated to have specific up-regulation of seven miRNAs located on the human 14q32–imprinted domain, the set included miR-127, miR-154, miR-154*, miR-299, miR-323, miR-368, and miR-370. In a large-scale genome-wide miRNA profiling study of patients with AML, miR-126/126* was found to be specifically over-expressed in both t(8;21) and inv(16) samples, whereas miR-224, miR-368, and miR-382 were almost exclusively over-expressed in the t(15;17) samples. Studies have also shown an association between the differential expression of miR-126/126* and the partial promoter demethylation status of the 287-bp CpG island. It was confirmed that miR-126 inhibited apoptosis and increased cell viability both in AML cells and mouse normal bone marrow progenitor cells, either alone or cooperated with the t (8;21) fusion gene. PLK2 was also verified to be a target of miR-126. Other studies have indicated that let-7b and let-7c were down-regulated in AML with t (8;21) and inv (16), this regulation might be associated with over-expression of RAS.

Several gene mutations have also been shown to play important roles in the generation of AML. Amongst them, the KIT, FLT3-ITD, and CEBPA mutations are the most frequently mentioned. Several studies have confirmed the characterization of AML by aberrant KIT tyrosine kinase activity. It has been found that miR-29b took part in a complex network related to the interacting transcription factors SPI (specificity protein1) and NF-κB as well as histone deacetylases, and leading to the over-expression of KIT. This autoregulatory loop eventually caused leukemogenesis. FLT3-ITD is often
viewed as a poor marker for prognosis in patients with AML, and miR-155 over-expression has consistently been reported in adult and paediatric patients with FLT3-ITD AML, which implied special links between them. Several trials have demonstrated that CEBPA triggers the expression of miR-223 in patients with AML. MiR-223 inhibited myeloid progenitor proliferation and promoted differentiation by targeting Mef2c and E2F1. This may partly explain why the down-regulation of miR223 and subsequent interference of myelopoiesis were often observed in patients with AML with mutated CEBPA. It was also pointed out that the CEBPA/miR-34a/E2F3 axis could be another mechanism of differentiation blocking in CEBPA-mutant AMLs (Table 1).

2) miRNAs as biomarkers in acute lymphoid leukaemia

In a large-scale genome-wide analysis of 17 cases of acute lymphoblastic leukaemia (ALL) and 52 cases of AML, four miRNAs were verified to discriminate ALL from AML with a diagnostic accuracy of 97–99%, amongst them, miR-128a and 128b were up-regulated and let-7b and miR-223 were down-regulated in ALL as compared with AML. In cases of paediatric ALL, miR-34a, miR-128a, miR-128b, and miR-146a were highly expressed, this was completely different from cases of AML, which were characterized by the up-regulation of miR-100, miR-125b, miR-335, miR-146a, and miR-99a. It was also observed that differences exist between cases of ALL with central nervous system (CNS) relapse and cases of ALL without CNS relapse. In cases of ALL with CNS relapse, miR-7, miR-198, and miR-663 were highly expressed and miR-126, miR-222, miR-551a, and miR-345 had a low level of expression compared to cases of ALL without CNS relapse. This finding can be used to predict the occurrence of CNS relapse in patients with ALL. Another study demonstrated that, when compared to normal CD34+ cells, 14 miRNAs in samples from patients with ALL, including miR-128a, miR-142-3p, miR-142-5p, miR-150, miR-151-5p, miR-181a, miR-181b, miR-181c, miR-193a, miR-30e-5p, miR-34b, miR-365, and miR-708, were up-regulated, whereas miR-100, miR-125b, miR-99a, miR-196b, and let-7e were down-regulated. This study also compared specific microRNA expressions amongst MLL, non-MLL precursor B-ALL, and T-ALL samples and showed that miR-708 was more predominantly expressed in the B-ALL sample and that miR-196b showed sharper increases in the T-ALL and MLL samples than in the B-ALL samples. The miR-17-92 cluster was typically found to be up-regulated in the ALL samples. It was suggested that dysregulation of the miR-17-92 cluster boosted the process of lymphomagenesis by repressing tumour suppressor genes. Bim and phosphatase and tensin homologue deleted on chromosome 10 (PTEN), two tumour suppressor genes, have been identified as the most likely targets of the miR-17-92 cluster. It has been suggested that down-

| Alteration type | miRNAs expression differences | Clinical application | References |
|-----------------|------------------------------|----------------------|------------|
| t(15,17)        | Up-regulated: miR-127, miR-154, miR-154*, miR-299, miR-323, miR-368, miR-370, miR-224, miR-382, miR-134, miR-376a, miR-126, miR-126*, miR-146a | Down-regulated: miR-17-3p, miR-185, miR-187, miR-194, miR-200a, miR-200c, miR-200d, miR-330, miR-339 | Patients with CN-AML and NPM1 mutations and no FLT3-ITD (NPM1 mutated /FLT3-ITD negative) are considered to comprise a molecular low-risk group that is similar to that of patients with core binding factor. AML: t (8;21) and inv (16). CEBPA mutations predicted better event-free, disease-free, and overall survival independently of other molecular and clinical prognosticators. Thus their related miRNAs also have significance in prognosis and may offer guidance regarding therapy. | 14,15,16 |
| t(8;21)         | Up-regulated: miR-126/126*, miR-146a | Down-regulated: let-7b, let-7c, miR-133a, miR-223 | 14,15,16 |
| inv(16)         | Up-regulated: miR-126/126*, miR-99a, miR-100, miR-224 | Down-regulated: let-7b, let-7c, miR-127 | 14,15,16 |
| KIT             | Down-regulated: miR-29b | 17 |
| FLT3-ITD        | Up-regulated: miR155, miR-10a, miR-10b, miR-511, miR-135a | Down-regulated: miR-143, miR-338, miR-30a-3p, miR-182, miR-145, miR-130a, miR-214, miR-203 | 16,18,19,23 |
| CEBPA           | Up-regulated: miR-181family, miR-335, miR-128, miR-192, miR-219-1-3p, miR-224, miR-340 | Down-regulated: miR-223, miR-196a, b, miR-149, miR-9, miR-21, miR-130b, let-7b, miR-194, miR-99b, miR-148a | 16,20,21,22,24 |
| NPMI mutation   | Up-regulated: miR-10a, miR-10b, miR-196a, miR-196b, let-7, miR-29a, b, c, miR-16-1 | Down-regulated: miR-204, miR-128a | 16,18 |
regulation of Bim mRNA and protein levels induced by miR-17-92 over-expression might contribute to lymphomagenesis and that the oncogenic activity of the miR-17-92 cluster was elevated by means of miR-19-mediated down-regulation of PTEN and further suppression of apoptosis (Table 2).

(3) miRNAs as biomarkers in chronic myeloid leukaemia (CML)

CML is characterized by the presence of the Philadelphia chromosome, which results from the translocation t(9;22) (q34;q11) and leads to a BCR-ABL fusion oncogene. The BCR-ABL oncoprotein recruits a tyrosine kinase and activates diverse related pathways, which leads to abnormal cellular proliferation and the inhibition of apoptosis. The BCR-ABL tyrosine inhibitor imatinib is the first-line drug treatment in patients with a new diagnosis of CML, and one of the most troublesome obstacles faced by doctors today is resistance to imatinib therapy. To distinguish imatinib-resistant patients from those with a response before clinical treatment, one study revealed a distinct signature consisting of 19 miRNAs that were differentially expressed between the two groups. Amongst them, miR-7, miR-23a, miR-26a, miR-29a, miR-29c, miR-30b, miR-30c, miR-100, miR-126, miR-134, miR-141, miR-183, miR-196b, miR-199a, miR-224, miR-326, miR-422b, and miR-520a were down-regulated, whereas miR-191 was up-regulated in patients with imatinib-resistant CML. This finding is of great significance in the selection of therapy for patients with a new diagnosis of CML.

(4) miRNAs as biomarkers in chronic lymphoid leukaemia

Chronic lymphocytic leukaemia (CLL) is the most common type of leukaemia in adults and accompanies multiple and recurrent chromosomal abnormalities. It has been reported that the down-regulation of the miR-15a/miR-16-1 cluster occurred in about 70% of patients with CLL and caused significant molecular damage. Klein constructed a transgenic mouse model to show that the DLEU2/miR-15a/miR-16-1 locus located in the minimally deleted region played an important role in controlling the expansion of the mature B cells by modulating proliferation. Its deletion accelerated the proliferation of B cells and was an important molecular lesion in patients with CLL. Previous studies have pointed out that the over-expression of these two genes (miR-15a and miR-16-1) could induce apoptosis by targeting BCL-2, an anti-apoptotic gene. Another study showed

| Items | MicroRNA | Expression in patients | Clinical significance | References |
|-------|----------|------------------------|----------------------|------------|
| ALL compared to AML | miR-128a, b, let-7b, miR-223 | Up-regulated, Down-regulated, Down-regulated | The specific miR expression of ALL can differentiate ALL from AML with a relative high accuracy. | 25 |
| CNS-relapsed ALL | miR-7, miR-198, miR-663, miR-126, miR-322, miR-551a, miR-345 | Up-regulated, Up-regulated, Down-regulated, Down-regulated, Down-regulated, Down-regulated | To predict the occurrence of CNS relapse in ALL. | 26 |
| Paediatric ALL | miR-34a, miR-128a, miR-128b, miR-146a | Up-regulated, Up-regulated, Up-regulated | Assist in diagnosing paediatric ALL. | 26 |
| ALL compared with normal CD34+HPCs | miR-128a, miR-142-3p, miR-142-5p, miR-150, miR-151-5p, miR-181a, b, c, miR-193a, miR-30e-5p, miR-365, miR-582, miR-708, miR-100, miR-125b, miR-99a, miR-196b | Up-regulated, Up-regulated, Down-regulated, Down-regulated, Down-regulated, Down-regulated, Down-regulated, Down-regulated | May function as significant biomarkers in diagnosing ALL. | 27 |
| B-ALL | miR-708 | Up-regulated | Might be useful in differentiating B-ALL, T-ALL, and MLL. | 27 |
| T-ALL | miR-196b | Up-regulated |
| MLL | miR-196b | Up-regulated |
that the over-expression of miR-16-1 suppressed cellular growth and cell cycle progression of human mantle cell lymphomas by targeting CCND1, thus preventing G1 to S-phase progression.37

By application of both cloning methods and analysis by quantitative real-time reverse-transcription polymerase chain reaction, Valerio identified five miRNAs with differential expression between the CLL samples and the controls. Amongst them, miR-21 and miR-155 appeared to be significantly over-expressed in almost every CLL sample analysed. It has been acknowledged that patients with CLL usually have different clinical courses, this clinical heterogeneity originates in part from the different mutational statuses of the immunoglobulin variable genes (IgVH), ZAP-70 expression, and specific cytogenetic alteration. In line with previous viewpoints, Valerio’s study found an over-expression of miR-150, miR-223, miR-29b, and miR-29c in the CLL cases with IgVH mutation as compared to those without IgVH mutation. As indicated previously, hypermutation of the IgVH genes often implied relatively stable disease, whereas an unmutated IgVH configuration accompanied a more aggressive clinical course. Thus the miRNAs mentioned above were closely associated with biological features containing known implications for prognostic factors and may prove to be useful in patient stratification.38

The significance of miRNAs in predicting the prognosis of leukaemia

In the diagnosis of a specific type of leukaemia, several parameters have been used to evaluate their prognosis, such as karyotype analysis, immunohistochemical analysis, and so on. Upon the discovery of various types of miRNAs in relation to haematopoietic malignancies, scientists have devoted themselves to studying the possibility of combining microRNA signature detection with the prevalent experimental practices in the prediction of the clinical prognosis of specific cases of leukaemia.

In a clinical trial of 86 patients with a new diagnosis of AML, the down-regulation of miR-96 in AML was associated with higher white blood cell counts and bone marrow blast counts and with lower haemoglobin and platelet counts, which reflected a strong relationship between miR-96 suppression and aggressive clinical features. The study also discovered that patients with low expression of miR-96 showed worse relapse-free survival rates and overall survival rates, which indicated a poorer prognosis for AML.40

High levels of MiR-335 expression were found in the bone marrow samples of paediatric patients with AML in several studies, and it was

| Items | MicroRNA | Expression in patients | Clinical significance | References |
|-------|----------|------------------------|----------------------|------------|
| Imatinib-resistant CML compared to Imatinib-responsive CML | miR-191 | Up-regulated | To select proper patients before taking imatinib into clinical therapy. | 34 |
| | miR-7 | Down-regulated | | |
| | miR-23a | Down-regulated | | |
| | miR-26a | Down-regulated | | |
| | miR-29a,c | Down-regulated | | |
| | miR-30b,c | Down-regulated | | |
| | miR-100 | Down-regulated | | |
| | miR-126 | Down-regulated | | |
| | miR-134 | Down-regulated | | |
| | miR-141 | Down-regulated | | |
| | miR-183 | Down-regulated | | |
| | miR-196b | Down-regulated | | |
| | miR-199a | Down-regulated | | |
| | miR-224 | Down-regulated | | |
| | miR-326 | Down-regulated | | |
| | miR-422b | Down-regulated | | |
| | miR-520a | Down-regulated | | |
| CLL | miR-15a/miR-16-1 | Down-regulated | These ectopic expressions are important molecular lesions and can be targets for therapy. | 35,36,37,38,39 |
| | miR-155 | Up-regulated | | |
| | miR-21 | Up-regulated | | |
| | miR-34a | Down-regulated | | |
| IgVH-mutated CLL | miR-150 | Up-regulated | Associated with biological features with known implications for prognostic factors and may prove to be useful in patient stratification. | 38 |
| | miR-223 | Up-regulated | | |
| | miR-29b, c | Up-regulated | | |
further demonstrated that a high serum level of miR-335 was correlated with the aggressive clinical features, and its up-regulation was most commonly observed in the FAB classification subtype M7 and associated with unfavourable cytogenetic risks.41 MiR-124-1 was frequently down-regulated in patients with AML, and it was confirmed that amongst the patients who received complete remission, those who had miR-124-1 under-expression had longer overall survival rates and relapse-free survival rates than those without miR-124-1 under-expression. This finding indicated that the down-regulation of miR-124-1 might have a favourable effect on the prognosis of AML.42

To determine the expression profile of paediatric ALL, Karolina collected samples from 51 children with diagnoses of ALL and found that increased expression of miR-128b expression and decreased expression of miR-223 could contribute to cell proliferation and survival, that the expression level of miR-223 would recover to a normal level during treatment, and that its down-expression could be a predictor of relapse. The study also found that increased expression of miR-128b correlated with a better prognosis, this finding would be useful in the pretreatment selection of patients with poor prognosis and might be of great significance in the adoption of more effective treatments.43

MiRNAs also present prognostic significance in CML samples. Edurne identified that miR-191, miR-29a, miR-422b, miR-100, miR-326, miR-26a were promising predictors of imatinib resistance in newly diagnosed CML patients.44 In another research, miR-17-92 cluster was discovered to be up-regulated in the chronic phase but not during blast crisis of chronic myeloid leukaemia, their expression decreased upon imatinib treatment in CML cell lines, indicating a control of the chronic phase by these miRNAs.45 In a study concerning the activities of miR-328 in myeloid cell differentiation and survival, Eiring reported that low expression of miR-328 in CML was associated with progression to the blast crisis phase of the disease.46

When analysing correlation between microRNA expression and CML prognosis, Stephane found that miR-142-3p correlated to the Sokal score, and the rapid inhibition of miR-18 during imatinib treatment was detrimental to an early haematologic remission, suggesting that miRNAs might serve as novel clinically useful biomarkers in CML47 (Table 4).

### The role of microRNA in therapeutic application

It has been said that all of the current efforts in the study of tumour are paving the way for the discovery of more effective treatments for the fight against fierce diseases that threaten the health of human beings. Therefore, the most promising clinical aspect of miRNAs is their application in targeted therapy. Because miRNAs can function as both oncogenes and tumour suppressors, their therapeutic directions can be divided into at least two groups: the use of miRNA mimics to restore the physiological expression of miRNAs that are down-regulated and the use of miRNA inhibitors targeted against over-expressed miRNAs.

1. Creation of miRNA mimics to re-express down-regulated miRNAs.

Many miRNAs can function as tumour suppressors, and their down-regulation is inevitably correlated with tumourigenesis. For example, let-7a plays an important role in the chemoresistance of AML cells, and a CXCR4/let-7a/BCL-XL axis may be responsible for this role. The activation of CXCR4 leads to the down-regulation of let-7a, which results in a high level of expression of an anti-apoptotic protein BCL-XL, this finding provides new ideas in the treatment of leukaemia given its correlation with leukaemia chemoresistance. Another experiment showed enhanced sensitivity to chemotherapy by transfection of pri-let-7a into AML cells, therefore, the restoration of let-7a to its normal expression level could be a promising direction in therapy for AML.48 MiR-9 has been demonstrated to act as a growth suppressor and differentiation inducer in t (8;21) AML through the inhibition of two oncogenes, LIN28B and HMGA2.

### Table 4 MiRNAs in relation to predicting prognosis of leukaemia

| Related miRNAs | Changes | Clinical use | References |
|----------------|---------|--------------|------------|
| MiR-96         | Down-regulated in AML | Correlates with aggressive clinical features, worse RFS, and OS | 40         |
| miR-335        | Up-regulated in AML   | Correlates with aggressive clinical features | 41         |
| miR-124-1      | Down-regulated in AML | Correlates with less RFS and OS | 42         |
| miR-128b       | Up-regulated in paediatric ALL | Correlates with better prognosis | 43         |
| miR-34a        | Down-regulated in CML | Correlates with poor prognosis | 39         |
| miR-328        | Down-regulated in CML | Correlates with progression to the blast crisis phase of the disease | 46         |
| miR-18         | Down-regulated during imatinib treatment | Correlates with early haematologic remission | 47         |

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Li and Zhong The diagnosis, prognosis, and therapeutic application of MicroRNAs

Hematology 2016 Vol. 21 No. 5268
which led to the restoration of the expression of let-7 and thus allowed the achievement of a greater knockdown of HMGA2. This might relieve leukaemia cells from their differentiation block and promoted apoptosis.\textsuperscript{49} The MiR-29 family could function as tumour suppressors in leukaemogenesis. It has been demonstrated that c-Myc induces the down-regulation of miR-29 family members, resulting in their targets, Akt2 and CCND2, which participated in the regulation of cell proliferation and cell apoptosis responsive up-regulation, and eventually led to AML development. That study also included the construction of the AML murine model to test the clinical effects of the miR-29 family by injection of viral particles that expressed miR-29 family members and showed a significant reduction of leukaemia cells in the bone marrow and the spleen as compared with the untreated ones.\textsuperscript{50} Oshrat found that miR-30e acted as a tumour suppressor by down-regulating the expression of BCR-ABL in CML, and the enforced expression of miR-30e in K562 cells suppressed proliferation and induced apoptosis of these cells and rendered them more sensitive to imatinib treatment.\textsuperscript{51} All of these conclusions remind us that miRNAs with aberrant expression represent potential therapeutic targets.

Many microRNA restoration techniques are undergoing clinical trials. The siRNAs and shRNAs are the hottest. It has been recognized that siRNAs resemble the mature miRNA duplex, and in fact, the two have been shown to be functionally substitutable against target miRNAs in several clinical trials. Therefore, the introduction of synthetic siRNA-like molecules that mimic the Dicer-processed miRNA duplex is a potential method for the rescue of under-expressed miRNAs.\textsuperscript{52} The major challenge today remains tissue-specific and cell-type-specific targeting, therefore future studies should focus on the invention of cell-specific siRNAs that target cell-surface receptors.\textsuperscript{53,54} The Pol III-driven shRNA system is another attractive method for the restoration of the expression of miRNAs because they can express the mature miRNA form, moreover, their well-defined transcription start and termination sites ensure abundant expression, which leads to effective target knockdown.\textsuperscript{55}

(2) Using interference-type strategies to silence up-regulated miRNAs

MiRNAs that function as oncogenes can be down-regulated by the use of interference-type strategies, which may be a novel field in the treatment of leukaemia. A previous study by Yamada proposed a model of miR-128a–mediated regulation of the Fas-mediated apoptosis pathway, in this study, he discovered that in Fas-resistant cells, demethylation of the promoter region of R3HDM1, which was the host gene of intronic miR-128a, led to the up-regulation of miR-128a following Fas stimulation and directly resulted in the delayed induction of apoptosis. This discovery shed light on settling leukaemia with resistance to chemotherapeutic problems, and antagomiR-128a might be adopted as a chemosensitizing agent, their combination with standard chemotherapy could be a new method for the treatment of patients with refractory leukaemia.\textsuperscript{56} It has been demonstrated that a high level of expression of miR-126 in AML was associated with a poor survival rate and higher chances of relapse, whereas the down-regulation of miR-126 expression induced apoptosis and decreased the clonogenic capacity of AML leukaemia stem cells (LSCs) and leukaemia progenitors without affecting the survival rate of normal bone marrow. This reminds us that miR-126 may be used as a therapeutic focus to specifically eradicate LSCs and therefore improve outcomes in patients with AML.\textsuperscript{57} To tackle such problems with over-expression, scientists have created several targeted strategies, including the use of anti-miRNA oligonucleotides, miRNA sponges, and miRNA masking.

Modified antisense oligonucleotides, also referred to as ‘antagomirs’ for their miRNA inhibition properties, are currently the most promising tools for miRNA intervention, and several studies have used different modifications of these antisense oligos to successfully inhibit miRNA expression in cell culture. Anti-miRNA oligonucleotides are a common type of antagomir, they are synthesized into single-stranded antisense oligonucleotides that consist of 17–22 nucleotides and inhibit the interaction between miRNAs and their diverse targets. Even so, anti-miRNA oligonucleotides have drawbacks regarding their inability to bind more than one miRNA.\textsuperscript{58} As another group of antagonists, microRNA sponges can bind to the targeted miRNAs at more than one point. They interact with the corresponding miRNA and prevent its interaction with the target mRNA.\textsuperscript{59} MicroRNA masking is another strategy to prevent miRNA-mRNA binding. In contrast to the methods described above, this technique binds directly to the target miRNA-binding site in the 3′-untranslated regions of its mRNA target, thus preventing miRNAs from binding to it and preventing the function of miRNAs.\textsuperscript{60}

(3) Other indirect strategies

Despite the two classic strategies discussed above, indirect methods, such as the use of epigenetic drugs like DNA-demethylating agents and histone deacetylase inhibitors, may also be of potential therapeutic use in the re-expression of epigenetically silenced miRNAs.

In a group of 353 patients with diagnoses of ALL, the expression of miR-124a was down-regulated by
hypermethylation of the promoter and histone modifications, thus inducing the up-regulation of its target, CDK6, and phosphorylation of retinoblastoma and leading to the abnormal proliferation of ALL cells. The use of CDK6 inhibitors could decrease ALL cell growth in vitro, whereas the over-expression of pre-miR124a led to decreased tumourigenicity in an in vivo mouse model.61 These findings exhibit the possibility of new therapeutic strategies for patients with ALL, either by the use of drugs that inhibit methylation and histone modifications or by directly targeting the CDK6 protein, and their combination may be a better choice.

Conclusions

The details discussed in this review demonstrate that miRNAs are not only involved in the normal process of haematopoiesis, but also play important roles in the pathogenesis of leukaemia. Their specific expression in leukaemia allows them to be used as novel biomarkers to differentiate the various types of leukaemia, to make more accurate diagnoses and furthermore, to estimate clinical prognoses before the development of an individualized treatment plan. The association between the ectopic expression of miRNAs and leukaemogenesis has already been viewed as a new avenue for treatment. However, because the technique is almost completely new, many obstacles still need to be settled before it can be put into practice, including the effective targeting of therapy, such as tissue-specific delivery, dosage and pharmacodynamics problems and several safety concerns such as those concerning off-target effects, RNA-mediated immunostimulation and the use of viral vectors. Moreover, some of the miRNAs may act as both oncogenes and tumour suppressors in different contexts. For example, the miR-17-92 cluster of miRNAs function in totally opposite ways in different tissues, and if it is not controlled well, targeted therapy in one tissue or system must aggravate abnormal function in others. This emphasizes the need for a better understanding of the expression patterns and potential roles of the expression of specific miRNAs in other tissues to avoid undesirable side effects. Once this field is exploited fully in the future, the overall survival rate and cure efficiency of leukaemia should improve.

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Contributors

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