microRNA Aberrations in Waldenström Macroglobulinemia

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Abstract

Waldenström macroglobulinemia (WM) is a low-grade B-cell lymphoma characterized by the presence of lymphoplasmacytic cells in the BM (BM) and monoclonal immunoglobulin M in the circulation. Although WM cells showed minimal changes in cytogenetic studies and gene expression analysis, primary WM tumor cells present with a micro-RNA (miRNA) signature that differentiates them from their normal counterparts. This may suggest the importance of miRNAs in supporting WM pathogenesis. Among deregulated miRNAs, miRNA-155 has been shown to play a pivotal role in the biological characteristics of this disease both in vitro and in vivo, thus providing the rationale for testing miRNA-based therapeutic approaches for the treatment of WM.

Introduction

MicroRNAs (miRNAs) constitute a class of small noncoding RNAs, described first in the nematode *Caenorabditis elegans*,[1] which act as negative regulators of gene expression by binding to the 3' untranslated region (UTR) of the target mRNAs, thus leading to gene translational repression.[2] The concept that mature miRNAs play a pivotal role in regulating development, cell differentiation, apoptosis, and cell proliferation has been widely accepted,[3] but they also play a crucial role in pathologic conditions, as demonstrated both in solid tumors and hematologic malignancies.[4-6] We have previously shown that miRNA-155 is upregulated in patients with Waldenström macroglobulinemia (WM) and that miRNA-155 represents an oncogenic miRNA in this disease: indeed miRNA-155 loss of function studies led to inhibition of WM tumor cell growth, both in vitro and in vivo. This was supported by the down-modulation of important prosurvival signaling pathways, such as Akt, extracellular signal–regulated kinase (ERK), and nuclear factor kappa B (NF-κB).[7] Specifically, WM cells that were miRNA-155 deficient presented with inhibition of phospho(p)-Akt, p-ERK, and NF-κB-p65 translocation from the cytoplasm to the nucleus, thus further confirming the oncogenic role of this miRNA. Importantly, it has also been demonstrated that miRNA-155 gain of function was responsible for lymphoblastic leukemia/high-grade lymphoma in transgenic mice, thus confirming the oncogenic role of miRNA-155 in other B-cell malignancies.[8-11]

Based on these observations, we tested the pharmacologic inhibition of miRNA-155 in WM by using locked nucleic acid (LNA) anti–miRNA-155. LNA represents a conformational analogue of RNA in which the ribofuranose ring in the sugar-phosphate backbone is locked in an RNA-like C3'-endo conformation. This results in high binding affinity between single-stranded LNA-modified anti-miRNA oligonucleotides and their complementary miRNA targets.[12] This approach has already been tested and showed inhibition of specific miRNAs of interest. In our studies, the use of LNA anti–miRNA-155 has been tested and showed an important antitumor effect in WM cells.[13]

Primary WM Cells Present with Overexpression of miRNA-155: Role of Anti–miRNA-155 in WM

We previously indicated that WM tumor cells present with higher expression of miRNA-155 compared with their normal cellular counterparts, as demonstrated in WM tumor cells isolated from 20 patients with WM compared with cells from healthy individuals.[7] This led us to hypothesize that miRNA-155 may act as an oncogenic miRNA in this disease. Therefore, we first evaluated miRNA-155 loss of function studies by knocking down miRNA-155 in WM cells, which led to inhibition of tumor cell growth in vitro, as indicated by inhibition of tumor cell proliferation. We next dissected the mechanisms that might have been responsible for this phenotype and found that by inhibiting miRNA-155 in WM cells, tumor cells presented with reduced activation of important prosurvival signaling pathways—such as mitogen-activated protein kinase (MAPK), Akt, and NF-κB—as shown at the protein level by using either Western blot or immunofluorescence studies. Also, miRNA-155 knock-down cells presented with reduced adhesion abilities and migration toward

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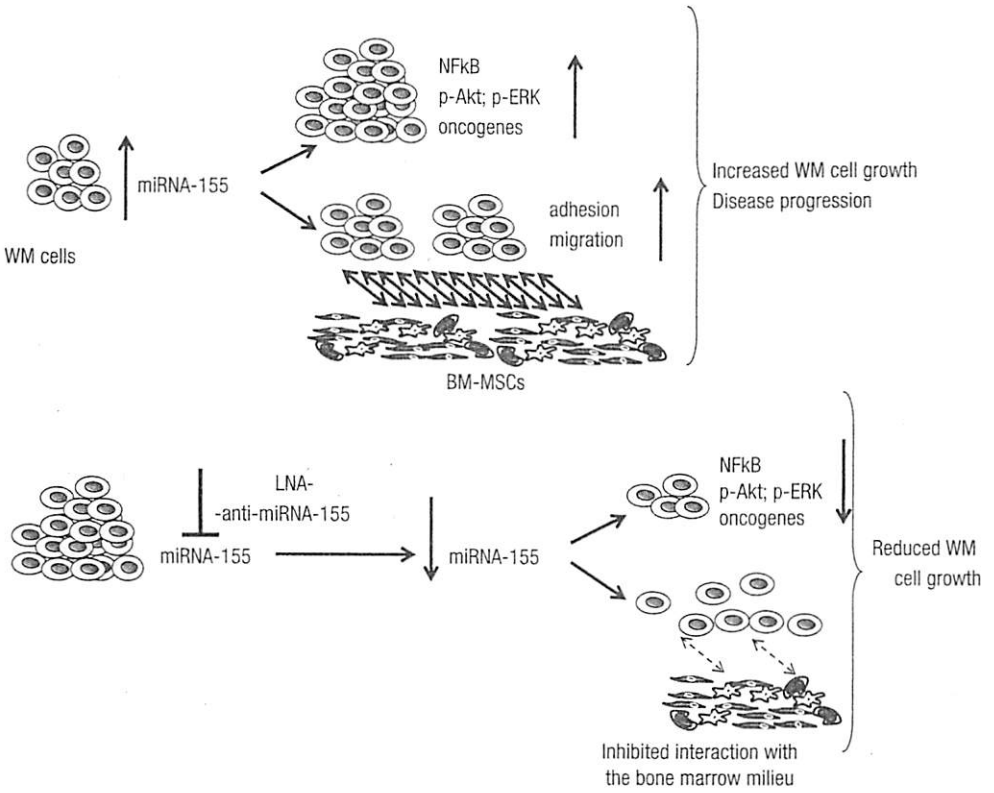
Figure 1 Oncogenic Role of miRNA-155 in Waldenström Macroglobulinemia: Rationale for miRNA-155 Therapeutic Approaches



stromal cell–derived factor 1 (SDF-1). We next confirmed these findings using in vivo models of WM tumor growth that showed antitumor effects in miRNA-155–deficient WM cells in mice.[7]

We conducted a new series of studies by testing an LNA anti–miRNA-155, with the main aim to show its anti-WM effect both in vitro and in vivo. In vitro data successfully indicated that antagonizing miRNA-155 in WM cells induced inhibition of WM cell proliferation. Importantly, new direct mRNA-155 targets were identified, and among those we found *CEBPB, SMAD5, SOCS1, MAFB, SHANK2,* and *SH3PXD2A*.[13] It is important to note that 6 genes were downregulated in WM cells compared with their related normal cellular counterparts. The functional role of these genes has been also evaluated in WM cells on treatment with the LNA anti–miRNA-55, and it was found that all the miRNA-155–targeted genes were indeed upregulated in WM cells. Overall, these findings indicate that the high level of the miRNA-155 in WM cells may partially explain the gene expression signature present in WM cells and that by antagonizing miRNA-155 using an LNA anti–miRNA-155 we may revert the genotype of WM cells to that of normal cells.

The use of the LNA anti–miRNA-155 was next evaluated in vivo, starting from the demonstration that it can be delivered after intravenous injections in vivo. Specifically, fluorescently labeled LNA anti–miRNA-155 appeared to be well distributed in the bone marrow (BM) niches, as well as in the spleen, where WM cells are usually centered and proliferate, leading to disease progression.[13] Based on these very promising findings, we also evaluated the actual anti-WM activity of the LNA anti–miRNA-155 using in vivo models, in which mice harboring WM disease were treated with LNA anti–miRNA-155 or the related control. The results indicated that this led to inhibition of WM tumor burden, as demonstrated by bioluminescence imaging, together with a significant reduction of WM cells within the BM niches, as well as within the spleen. In the control mice, the WM disease showed signs of progression both in the BM and in the spleen. Because of the previous data indicating direct targets of miRNA-155 for *CEBPB, SMAD5, SOCS1, MAFB, SHANK2,* and *SH3PXD2A*, the expression of these same genes were tested in mice treated with LNA anti–miRNA-155 and compared with control mice; upregulation of those genes was demonstrated in the BM of mice that received LNA anti–miRNA-155 treatment compared with control mice.[13] This represents further proof of the efficacy of LNA anti–miRNA-155 to target miRNA-155–related targets in vivo.

It has been well accepted and established that the BM milieu is crucial in supporting the pathogenesis of B-cell malignancies, and this is true for multiple myeloma, chronic lymphocytic leukemia, and WM as well.[14-16] Therefore, it has been crucial to test the efficacy of LNA anti–miRNA-155 in WM in the presence of BM mesenchymal stromal cells (BM-MSCs) isolated from patients with

WM: What researchers observed is that despite the prosurvival effect of the BM-MSCs on the clonal WM cells, the LNA anti–miRNA-155 was able to reduce WM cell proliferation, thus overcoming the BM-MSC protective effect on WM cells. To better clarify these findings, BM-MSCs obtained from miRNA-155 knockout mice were used, and although wild-type murine BM-MSCs were able to increase WM proliferation in a coculture system, the same was not observed when WM cells were in contact with miRNA-155–deficient murine BM-MSCs, indicating the importance of miRNA-155 even at the level of BM niches.[13]

Conclusions

MiRNA expression patterns have been useful in delineating biological alterations as well prognostic factors in solid and hematologic tumors. WM is an example of B-cell malignancy in which miRNA aberrations have been shown to be crucial in supporting WM pathogenesis and disease progression. We have previously characterized the miRNA signature in primary WM cells compared with the related normal cellular counterparts and showed that miRNA-155 acts as an oncogenic miRNA in this disease (Figure 1), thus supporting the rationale for targeting miRNA-155 in WM cells. We therefore tested the LNA anti–miRNA-155 to selectively target and inhibit miRNA-155 in WM cells, together with miRNA-155–targeted genes, and demonstrated that LNA anti–miRNA-155 significantly inhibits miRNA-155 levels in WM cells, leading to inhibition of WM tumor growth both in vitro and in vivo. Overall, these findings provide the preclinical rationale for targeting miRNA-155 in WM as well as in other B-cell malignancies harboring miRNA-155 overexpression.

Disclosure

The authors have stated that they have no conflicts of interest.

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