Epigenomics in Waldenström's macroglobulinaemia

Antonio Sacco, Adriano Fenotti, Stefano Bazzana, Luisa Imberti, Giuseppe Rossi, Christopher J. Patterson, Steven P. Treon, Irene M. Ghobrial, Aldo M. Roccaro

A S S T Spedali Civili, Department of Medical Oncology, CREA Laboratory, Brescia, Italy
ASST Spedali Civili, SITRA, Brescia, Italy
ASST Spedali Civili, Collegio IPASVI, Brescia, Italy
Dana-Farber Cancer Institute, Department of Medical Oncology, Boston, MA, USA

Keywords:
Waldenström's macroglobulinaemia
Epigenomic
Histone acetylation
microRNAs

A B S T R A C T

Epigenomics refers to study of the epigenome, which represents changes in gene expression that are not induced by DNA sequence aberrations. For instance, DNA methylation, histone acetylation and microRNAs may modulate gene expression without altering the gene sequence. Waldenström's macroglobulinaemia (WM) is a low-grade B-cell lymphoma, classified as lymphoplasmacytic lymphoma, characterized by the presence of clonal lymphoplasmacytic cells in the bone marrow and serum monoclonal immunoglobulin-M in the circulation. It is a rare disease and, although indolent, it remains incurable with a median overall survival of 5–6 years. Most patients succumb to disease progression. WM cells present with aberrant histone hypoacetylation that may be explained, at least in part, via deregulated microRNAs, thus suggesting the use of histone deacetylase inhibitors or microRNA-based therapies in this disease.

© 2016 Elsevier Ltd. All rights reserved.
Introduction

Epigenetics was first defined by Conrad Hal Waddington (1905–1975), a British developmental biologist, paleontologist, geneticist, embryologist and philosopher, as ‘the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being’ [1,2]. This definition has since been modified to changes in gene expression that are not due to any alteration in the DNA sequence [3]. microRNAs (miRNAs) and histone acetylation represent epigenetic markers that may support tumour progression, as demonstrated in B-cell clonal disorders such as Waldenström’s macroglobulinaemia (WM) [4]. Indeed, bone-marrow-derived WM cells present with reduced histone acetylation compared with their normal cellular counterparts. Studies have also described the potential role of miRNAs in mediating the histone acetylation status in WM cells where a specific miRNA signature has been identified [5]. miRNAs act as negative regulators of gene expression, and their functional role has been demonstrated in both physiological and pathological conditions; for instance, miRNAs modulate development, cell differentiation, apoptosis and cell proliferation [6–8]. miRNAs also play a crucial role in supporting tumour pathogenesis, as documented in both solid tumours and haematological malignancies, including WM [4,5,9]. Specifically, recent evidences indicate that miRNAs may act as modulators of histone acetylation within WM cells [4,5]. Overall, these findings provide the preclinical rationale for using histone deacetylase inhibitors and miRNA-based therapeutic strategies for the treatment of patients with WM.

miRNA profiling in WM cells

CD19+ selected tumour cells obtained from the bone marrow of patients with WM present with a peculiar miRNA profile that distinguishes WM clonal cells from their normal cellular counterparts isolated from the bone marrow of healthy subjects [5]. Overall, WM cells show upregulation of miRNA-155, -206, -494, -363*, -184 and -524-3p, and downregulation of miRNA9*. While tumour suppressors and inhibitors of cell cycle progression are among the targets of the increased miRNAs, transcription factors and oncogenes are among the targets of the reduced miRNAs, thus suggesting the role of miRNAs in mediating inhibition of tumour suppressors and activation of oncogenes, leading to increased WM cell growth and enhanced tumour progression. miRNA-155 has been shown to act as an onco-miRNA in several lymphoid malignancies, such as chronic lymphocytic leukaemia and diffuse large B-cell lymphomas [10,11]. miRNA-155 has also been confirmed to be an onco-miRNA in WM, as supported by in-vitro and in-vivo studies that demonstrated its role in modulating WM cell growth and WM cell dissemination in vivo [5]. miRNA-155-silenced WM cells show upregulation of cyclin-dependent kinase inhibitors (p18, p19, p21, p27), and downregulation of cyclin-dependent kinases (cdk-2, -4, -6) and cyclins (D1, D2, D3). In addition, miRNA-155-silenced WM cells present with inhibition of pro-survival pathways, such as MAPK/ERK, AKT and AKT-downstream targeted proteins [5]. miRNA-155-silenced WM cells also show inhibited WM cell proliferation, and reduced WM cell adhesion and migration. These in-vitro findings were further confirmed using in-vivo models, where reduced WM tumour growth and WM homing to the bone marrow were inhibited in miRNA-155-silenced cells compared with normal control cells [5]. Of note, lock nucleic acid (LNA)-based anti-miRNA-155 therapies recapitulate the anti-WM effect using both in-vitro and in-vivo models, thus suggesting the use of LNA-anti-miRNA-155 as a novel anti-WM therapeutic strategy [12].

miRNA and histone acetylation status in WM cells

Among other deregulated miRNAs, miRNA-206 and miRNA-9* are increased and decreased, respectively, in WM cells, compared with cells derived from healthy individuals [4]. Of note, miRNA-206- and miRNA-9*-predicted targets include histone acetyl transferases (HATs) and histone deacetylases (HDACs), respectively. This prompted investigators to hypothesize that miRNA-206 and miRNA-9* may be involved in mediating histone acetylation in WM cells [4]. A well-balanced homeostasis of nucleosomal histone acetylation is essential for the regulation of gene transcription; specifically,
Histone hypoacetylation or hyperacetylation is responsible for gene transcription inhibition and activation, respectively [13,14]. A balanced histone acetylation status is maintained through tight regulation of both HDAC and HAT levels. The occurrence of increased HDAC levels may characterize tumour cells leading to uncontrolled cell growth [15–19]. For instance, it has been reported that HDACs may repress the transcription of cyclin-dependent kinase inhibitors and pro-apoptotic factors, thus favouring cell proliferation and cell survival [20,21]. Moreover, it has been reported that HDAC1 may repress the tumour suppressor p53; it may also be responsible for inducing vascular endothelial growth factor, thus enhancing angiogenesis [22,23].

Imbalance between HDACs and HATs may occur during malignancies, including both solid tumours and haematological malignancies [24,25]. Recent reports suggest that miRNAs play a role in interfering with the epigenetic machinery, leading to modifications of histone acetylation [24–27]. In the specific context of WM, bone-marrow-derived tumour cells present with enhanced expression of HDACs and reduced levels of HATs compared with their normal cellular counterparts [4]. In addition, evidence has confirmed the role of miRNA-9* and –206 in modulating histone acetylation status in WM cells; specifically, restoring the reduced level of miRNA-9* and inhibiting the increased level of miRNA-206 favoured acetylation of histone-H3 and -H4 in WM cells. Moreover, the overexpression of miRNA-9* is responsible for reducing the proliferation and increasing the toxicity of WM cells [4].

Targeting HDACs in WM

Based on preclinical studies demonstrating aberrant HDAC activity in WM cells, a phase 2 trial using the HDAC inhibitor, panobinostat, as a single agent, was initiated in WM patients with relapsed/refractory disease [28]. A minimal response or better was obtained in 47% of patients (22% partial remissions, 25% minimal responses). In addition, stable disease was documented in 50% of the patients and, importantly, none of the patients showed progression while on therapy. The median time to first response was 1.8 months (range 1.7–3.2 months). The median progression-free survival was 6.6 months (90% confidence interval 5.5–14.8 months). Grade 3 and 4 toxicities included thrombocytopenia (67%), neutropenia (36%), anaemia (28%), leukopenia (22%) and fatigue (11%). Overall, this study demonstrated that panobinostat represents an effective and safe regimen for WM patients with relapsed/refractory disease, thus establishing an important role for HDAC inhibition as a novel therapeutic approach for the treatment of WM [28].

Conclusions

Aberrant acetylation may occur in cancers where the disruption of either HATs or HDACs may play a crucial role in supporting tumour pathogenesis. Genes encoding for HATs may be overexpressed as the result of translocation, amplification or mutation, as demonstrated in both solid tumours and haematological malignancies [29–32]. Similarly, HDACs may also favour tumour transformation and disease progression due to their ability to support the function of oncogenes resulting from specific translocations in certain types of leukaemias or lymphomas [33,34]. Moreover, HDACs may also repress pro-apoptotic factors or negative regulators of cell cycle progression, thus inducing cell proliferation and enhancing cell survival. In the specific context of WM, it has been shown that tumour cells present with enhanced HDAC activity, and this may be due, at least in part, to the deregulation of miRNA-9* that has been proven to facilitate expression of HDAC-4 and -5, thus recapitulating the importance of epigenetics in supporting the progression of WM. Taken together, these findings have led to the initiation of clinical trials that have confirmed the importance of HDAC inhibition for the treatment of WM patients with relapsed/refractory disease. Finally, WM cells also present with aberrantly expressed miRNAs, and preclinical studies have provided the rationale for testing LNA-anti-miRNA-155 or pre-miRNA-9* as novel therapeutic strategies in WM.
Practice points and research agenda

- The evidence for histone hypoacetylation in WM cells and the demonstration of HDAC-inhibitor-dependent induction of cytotoxicity in WM cells have provided the rationale for using HDAC inhibitors in WM. Several clinical trials are using HDAC inhibitors in combination with other anti-WM agents for the treatment of patients with WM.
- The evidence for miRNA-155-overexpression in WM cells and the in-vivo and in-vitro demonstration of anti-miRNA-155-dependent induction of an anti-tumour effect in WM have provided the preclinical rationale for using LNA-anti-miRNA-155-based therapies in WM.

Conflict of interest statement

None declared.

Acknowledgements

Associazione Italiana contro le Leucemie, Linfomi e Mieloma, AIL Brescia; International Waldenström’s Macroglobulinemia Foundation.

References


