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REVIEW ARTICLE

Circulating MicroRNAs in Multiple Myeloma: a Literature Review

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SUMMARY

Multiple myeloma is a tumour of antibody-secreting plasma cells characterized by clonal expansion and accumulation of monotypic plasma cells in the bone marrow. It is an incurable malignant neoplasm accounting for 10% all hematological malignancies. Globally, the annual percentage of new cancer cases and deaths attributed to multiple myeloma is estimated at about 0.8% and 1%, respectively. Furthermore, its global incidence ranges from 0.5 - 12/100,000 population. It causes hypercalcemia, renal insufficiency, anemia, thrombocytopenia, leucopenia, bone lesions, bone fractures, spinal stenosis, and end-organ damages. This neoplasm occurs due to a complex cytogenetic and chromosomal aberrations. These aberrations affect the expression and functions of microRNAs. Abnormal expression of these microRNAs plays an important role in the pathogenesis and angiogenesis of multiple myeloma and could have a potential role in the diagnosis, prognostic stratification, and treatment of multiple myeloma. This review aimed at summarising the expression of microRNAs and the implication of their dysregulation in the pathogenesis, diagnosis, and treatment of multiple myeloma.
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KEY WORDS

microRNAs, multiple myeloma

LIST OF ABBREVIATIONS

BCL - B-Cell Lymphoma Protein
BM - Bone Marrow
BMSC - Bone Marrow stromal cell
FGFR - Fibroblast Growth Factor Receptor
HIF - Hypoxia Inducible Factor
JAK - Janus Kinase
MCL - Myeloid Leukemia Cell Differentiation Protein
MGUS - Monoclonal Gammopathy of Undetermined Significance
miRNA - MicroRNAs
MM - Multiple Myeloma
MMP - Matrix Metallopeptidase
MMR - Mismatch Repair
MMSET - Multiple Myeloma Set Domain
mRNA - Messenger RNA
NF-$_K$B - Nuclear Factor Kappa B
PC - Plasma Cell

RAS - Rat Sarcoma
RISC - RNA-Induced Silencing Complex
RNA - Ribonucleic Acid
SMM - Smoldering Multiple Myeloma
STAT-3 - Signal transducer and activator of transcription 3
UTR - Untranslated Region
VEGF - vascular endothelial growth factor

INTRODUCTION

Background

Multiple myeloma (MM) is a tumor of antibody-secreting plasma cells (PCs) characterized by clonal expansion and accumulation of monotypic PCs in the bone marrow (BM) [1,2]. It is a heterogeneous malignancy with complex cytogenetic abnormalities, comprising the presence of hypodiploidy, gene mutations, chromosome aberrations, duplications and deletions of chromosomes. These cytogenetic abnormalities dysregulate the expression of miRNA including its binding sites. The dysregulation of miRNA affects the normal function and leads to pathological processes in oncogenic or tumor-suppressing actions of the target genes via the activation of multiple signalling transduction pathways [3]. MM is a currently incurable PC proliferative disorder that results in considerable morbidity and mortality [4]. This neoplasm represents the second most common hematologic disease accounting for more than 10% of all hematologic malignancies. It causes about 1% of neoplastic diseases [5]. Myeloma is always preceded by an asymptomatic premalignant stage called monoclonal gammopathy of undetermined significance (MGUS). MGUS often remains stable for years without showing any clinical symptoms. However, for unknown reasons, the benign condition can progress into indolent MM and smouldering MM (SMM) at a rate of 1% per year [6,7]. SMM is symptomatic and meets diagnostic criteria of MM like an elevated concentration of M-protein, percentage marrow PCs, and end-organ damage defined by hypercalcemia, renal insufficiency, anemia, and bone lesions [8]. In SMM, there are no related organ or tissue impairments that differ from MM. MM is the last stage and characterized by various cytogenetic abnormalities. There is no single factor that identifies MGUS patients who are likely to progress to myeloma, and patients need to be monitored at regular intervals [9].

Globally, MM is one of the most common challenging hematologic malignancies. It develops due to complex cytogenetic abnormalities and chromosomal aberrations. Its burden includes around 103,826 (0.8%) new cases and 72,453 (1%) deaths annually due to this untreatable neoplasm [10]. The incidence of MM in Asia ranges from 0.5 - 1/100,000, whereas in Africa and America it reaches 10 - 12/100,000. This problem is still untreatable and its burden is rising in the world due to its initial asymptomatic stage like MGUS making it difficult for early intervention. It also causes hypercalcemia, renal insufficiency, anemia, thrombocytopenia, leucopenia, bone lesion, bone fractures, spinal stenosis, and end-organ damages [11]. Hypercalcemia can cause life-threatening dehydration and renal failure. Bone-related disorders account about 70% of patients diagnosed with MM. It is often undetected until the late course of the disease because patients experience no symptoms despite the bone damage. Recent studies indicated that circulating microRNAs (miRNAs) are important as stage differentiation, diagnostic, and prognostic biomarkers and as a therapeutic alternative for MM.

miRNAs play crucial roles in the pathogenesis of MM, either in tumour-suppressing or oncogenic functions, depending on their target genes [12]. In most circumstances, MM occurs due to chromosomal translocations, which influence the regulation and expression level of miRNAs. The aim of this review is to describe the expression of miRNAs and the implication of their dysregulation in the pathogenesis, prognosis, diagnosis, and treatment of MM. In the early stage of the disease, MM is without evidence of any metabolic disturbance and slowly progresses to SMM. SMM is symptomatic including disturbances of hematological parameters like leucopenia, thrombocytopenia, and anemia. The gold standard diagnosis of this disorder is BM examination, which is a painful and technically difficult sample to obtain. However, circulating miRNAs in the serum or plasma are stable and simple and could have the potential to be diagnostic, prognostic, and therapeutic biomarkers of MM, encouraging us to conduct this review.

MATERIALS AND METHODS

A review was conducted on the basis of relevant literature on the current topic retrieved from electronic databases such as PubMed, PubMed Central, Scopus, Science Direct, Google Scholar, and Google. Articles on the current issue were searched using keywords and phrases such as ‘MicroRNAs’, ‘MiRNAs’, ‘Myeloma’, and ‘Multiple Myeloma’ separately and in combination. Articles such as reviews, systematic reviews, and meta-analyses were also used while writing this document. The current review included peer-reviewed original and review articles published in English language.

miRNA biogenesis and regulation

miRNAs are part of a family of non-coding, small (20 - 25 nucleotides) single-stranded ribonucleic acid (RNA) molecules that regulate messenger ribonucleic acid (mRNA) translation, stability, and degradation [13]. They are small, evolutionarily conserved molecules that bind to target mRNA to prevent protein production by one of two distinct mechanisms [14]. The first mechanism is binding to the 3′ end of the untranslated region (UTR) of the target mRNAs with partial sequence complementarity. The binding to target genes represses the target mRNA genes, consequently, translation is inhibited. The second mechanism is through the RNA interfer-

ence which results in the degradation of mRNA at a post-transcriptional level. Mature miRNAs play a pivotal role in regulating physiologic processes such as development, cell differentiation, apoptosis, response to stress, and cell proliferation. The above functions of mature miRNAs might be dysregulated due to chromosomal abnormalities in different hematological neoplasms including MM [15-18].

The maturation of miRNAs starts through the transcription into primary miRNAs that can be facilitated by RNA polymerase II or III in the nucleus. Then, the primary-miRNA transcript is stabilized by 5′ capping and 3′ polyadenylation. It is then processed into precursor miRNA by Drosha and Pasha. A precursor-miRNA (60 - 80 nts) forms a hairpin or stem-loop structure, followed by export into the cytoplasm through Ran-GTP-dependent exportin 5. In the cytoplasm, the precursor-miRNA is further processed by RNase III Dicer into mature miRNA duplex (22 - 25 nts), which will then be loaded into an RNA-induced silencing complex (RISC) [15,19,20].

The functionally mature miRNA strand of the duplex is retained in the RISC for recognizing the mRNA target through sequence complementarity between the miRNA seed region and the 3′ UTR of the target gene. The target gene is inhibited by either translational inhibition or mRNA degradation mediated by the miRNA-associated RISC shown below (Figure 1). More than one miRNA can bind to one transcript at a time. Each miRNA can target hundreds of transcripts, either by binding of their seed sequence (i.e., the 2nd to 7th nucleotides from the 5' end of the mature miRNA) to the 3'-UTR of their target mRNA directly, or indirectly targeting another regulator protein. Precise miRNA expression is essential for normal cellular functions, including apoptosis, proliferation, and differentiation. Conversely, dysregulation of miRNA expression is implicated in various malignant diseases, including MM [16,17].

miRNAs can specifically bind to targeted mRNA and suppress protein production. According to bioinformatics analysis, more than 60% of protein-coding genes may be targeted by miRNAs. miRNAs have been shown to play a fundamental role in diverse physiological and pathological processes that include cell development, cell differentiation, apoptosis, stress response, and fat metabolism through the regulation of gene expression [21].

Expression and role of miRNAs in MM

Individual miRNAs can target multiple mRNAs and control transcription in approximately one-third of human genes. However, this function is altered in MM due to the presence of complex cytogenetic and chromosomal aberrations. There are over two thousand miRNAs in the human genome, and most of them have unknown functions. Some of the known miRNAs have many roles in MM like oncogenic and tumour-suppressing roles and biomarkers for the diagnosis. Others are also involved in the pathogenesis, angiogenesis, and targeted therapy for MM [22-24].

Role of microRNA in the pathogenesis of MM

MM is a chronic, still incurable disease characterized by a clonal proliferation of malignant PCs. Numeric chromosomal abnormalities, translocations, gene mutations, epigenetic alterations, and direct interactions between malignant PCs and stromal cells in the BM microenvironment are all involved in the pathogenesis of MM [25].

The pathogenesis of MM begins with chromosomal translocations of 14q32.33 and 4p16.3 resulting in the deregulation of the Multiple Myeloma Set Domain (MMSET) and Fibroblast Growth Factor Receptor 3 (FGFR3) genes. These early chromosomal alterations are observed in both MM and MGUS. As the disease progresses, secondary chromosomal aberrations and extra gene mutations including activation of the Rat Sarcoma (RAS) pathway, complex karyotype abnormalities in the MYC gene, mutations in the TP53 and FGFR3 genes, and inactivation of cyclin-dependent kinase inhibitor 2A and cyclin-dependent kinase inhibitor 2C also lead to complex cytogenetic abnormalities. The above genetic abnormalities result in the alteration of the expression level of miRNAs and adhesion molecules on malignant PCs and consequent abnormal interactions between PCs and bone marrow stromal cell (BMSC) compartment. Signals from the BM niche grant a viable environment for MM cell growth and survival. This type of driven microenvironment gives the supporting role for the interaction of MM cells and BM components. Effects of miRNAs regulate the close interaction between MM and BM microenvironments. Dysregulation of miRNA expression due to chromosomal aberrations may contribute to dysregulated expression of target genes in MM. Translocation of chromosomal fragments or deleted chromosome segments could lead to either reduction or complete loss of some miRNA's expression, or to enhanced expression of other miRNAs that could down-regulate the expression of a protein needed for the transcription of another miRNA [26]. In support of this, a study done by Lijuan Chen et al. showed that deletion of chromosome 13 [del(13q14)] leads to loss of miRNA-17, miRNA-18, miRNA-19a, miRNA-19b-1, miRNA-20, and miRNA-92-1 resulting in the activation of the MYC proto-oncogene [27].

MYC is an oncogenic transcription factor that regulates human target genes to promote cell proliferation and apoptosis. However, this MYC gene is controlled by the above-listed miRNAs. Due to loss of miRNAs, the MYC gene is activated, which leads to activation of different signaling pathways including the Nuclear Factor-Kappa B (NF-κB) signaling pathway [28], Interleukin 6 (IL6) signal transducer and activator of transcription 3 (STAT-3) signaling pathway [29], tumour protein P53 (P53)/mouse double minute 2 homolog signaling path-

Table 1. A summary of dysregulated miRNAs: their role and implications in MM.

Dysregulated miRNA during MM	The potential implication of deregulated miRNA	Ref.
miRNA-765	Oncogenic role	[51]
miRNA-20a	Oncogenic role in MM	[34]
miRNA-148a, miRNA-181a, miRNA-20a, miRNA-221, miRNA-625, miRNA-99b	Upregulatation of MM cells	[52]
miRNA-21 and miRNA-106b25 cluster	Stage differentiation (MDUS) Oncogenic role Promoting PC transformation and survival by blocking apoptosis, and predisposing to secondary genetic abnormalities	[13]
miRNA-17~92, along with miRNA-21	Blocks apoptosis and promotes cell survival	[13]
miRNA-181a, and miRNA-32	P53 positive regulator	[13]
miRNA-19	Negative regulator SOCS-1 PC transformation and oncogenesis through STAT-3, impacting apoptosis regulators such as the Bcl-2 family members	[53,54]
miRNA-152, miRNA-15a, miR34a, and miRNA-223	Downregulation of MM cells by blocking of dick-Kopf homolog 1 family of proteins transcriptional activity by binding to the 3′ UTR of mRNA Plays a potential for developing newer therapeutic strategies for MM	[55]
miRNA-1271	Inhibited proliferation promoted apoptosis of MM cells	[56]
miRNA-29a, miRNA-29b and miRNA-29c	Have significant tumor inhibitory effect that inhibits the activation of STAT-3 by binding with Janus Kinase and decreased proliferation of MM cells via the suppression of the transcription of downstream genes. It also increases MM cell apoptosis (BCL2) and has potential in clinical application as a micromolecular nucleic acid drug for MM therapy.	[3]
miRNA-29b	Therapy of MM	[57]
miRNA-194	Therapy by inhibiting cell growth and promotes apoptosis of MM cell lines in a P53 dependent manner	[33]
miRNA-29a	Upregulation of MM Discriminate new MM patient from a healthy donor Initiation and monitoring of chemotherapy	[19]
miRNA-16	Upregulation of MM	
miRNA-155	Downregulation of MM	
miRNA-221/222	The expression level of these miRNA is high in MM t(4;14) and silencing of miRNA-221/222 by specific inhibitors could result in a powerful antitumour activity in MM cells via strong suppression of p27. Therefore, silencing miRNA-221/222 plays a major role for diagnostic, prognostic, and therapeutic biomarkers for MM patients	[58]
miRNA-744, miRNA-130a, miRNA-34a, let-7d, let-7e	Prognosis, diagnosis, and stage differentiation between MGUS and MM	[37]

way [30], and phosphatidylinositide 3-kinases (PI3K)/ protein kinase B (AKT) signaling pathway [3]. On the other hand, miRNAs play a major role in silencing gene expressions. This might be done by regulating its expression at the genome level. One of the gene silencing mechanisms is DNA methylation, which occurs at the promoter region that is encoded by miRNAs. In cancer cells, methylation of the promoter-associated CpG island of a tumor suppressor protein-coding or miRNA is associated with heterochromatin configuration and therefore gene silencing takes place. In normal cells, a majority of genes with promoter-associated CpG islands are usually unmethylated, associated with euchromatin configuration, and hence are generally transcriptionally active. Methylation occurs at the promoter region of CpG island. This leads to recruitment of histone methyltransferase, methyl-CpG-binding domain protein, and histone deacetylase resulting in the formation of a com-

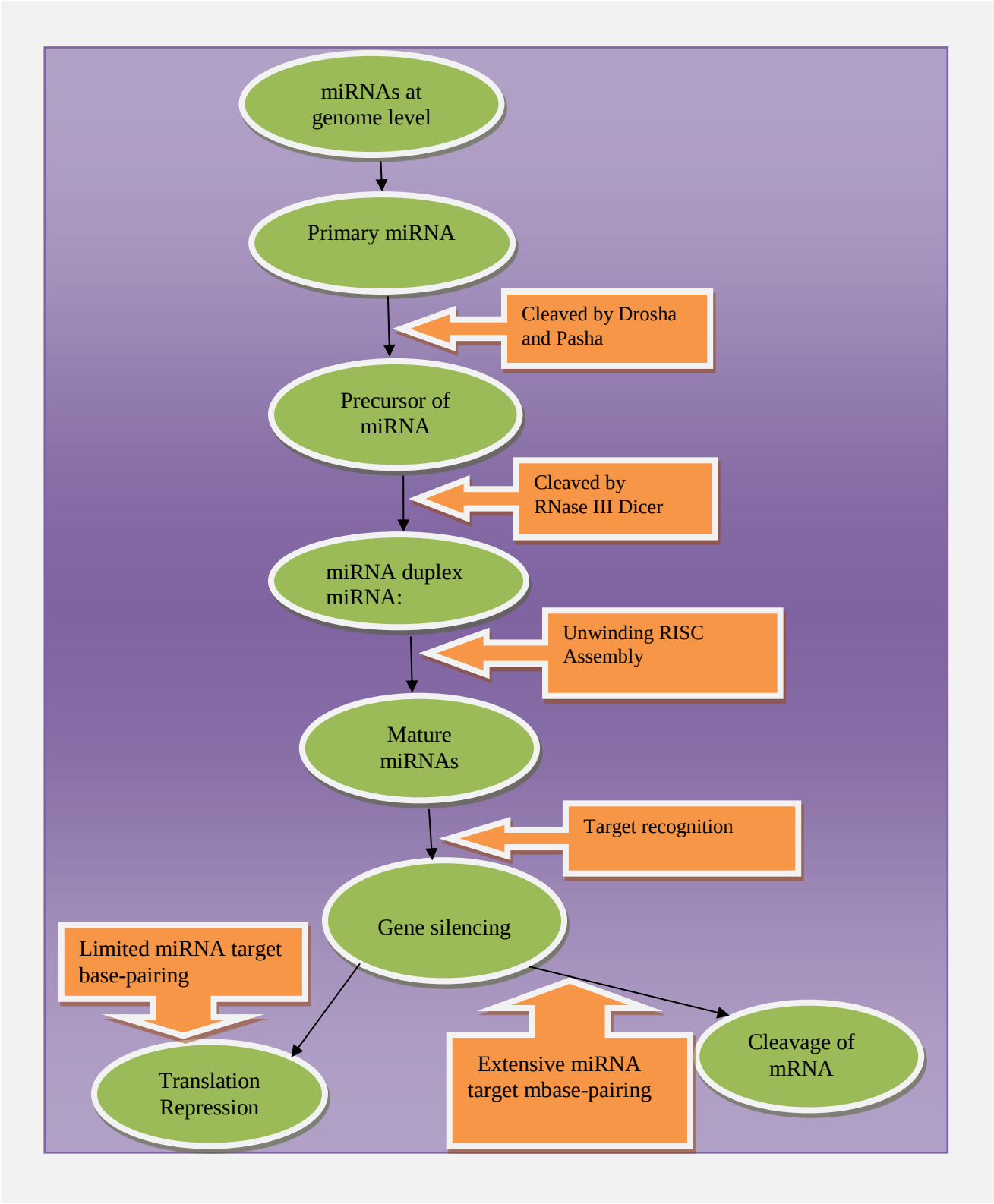

Figure 1. MicroRNA biogenesis, maturation, and functions.

pact heterochromatin configuration, which precludes the binding of transcription factor complex, and therefore, silencing of the associated gene [31,32]. In MM, by genome-wide or gene-specific approaches, aberrant DNA methylation has been found to mediate the loss of a number of miRNAs which are important to regulate cell

cycle progression, cell signalling or apoptosis, including cyclin-dependent kinase inhibitor 2A, cyclin-dependent kinase inhibitor 2B, death-associated protein kinase and suppressor of cytokine signalling. Thus, different mechanisms could lead to aberrant expression of miRNAs and their involvement in MM pathogenesis [1,31].

Oncogenic and tumor suppressor role of miRNAs in MM

miRNAs have multiple roles in the development of MM, as they are capable of modulating oncogenic, tumour suppressor, and different pathways including c-MYC, p53, and RAS pathways. miRNAs have also been shown to target p53 and/or components of p53 regulatory pathways, thereby directly and/or indirectly affecting its activities. The previous study showed that miRNAs which are directly transactivated by p53 like miRNA-34a and miRNA-194 are distinctly associated with P53 activations [4]. A similar study conducted by Corthals et al. showed that miRNA-21, miRNA-19a, and miRNA-19b are involved in upregulation of myeloma PCs through activation of the signal transducer and activator of transcription 3 (STAT-3) and IL6 antiapoptotic pathway. Additionaly, cytogenetic analysis like deletion 13q14, t(4;14), t(11;14), t(14;16) showed that miRNA-122a, miRNA-33, miRNA-489, miRNA-519e, and miRNA-555 were abnormally dysregulated and upregulate MM cells [33].

The other miRNA having oncogenic roles is miRNA-20a, which is a member of the miRNA-17-92 cluster located on chromosome 13. This miRNA is elevated in MM and the mechanism is mediated by altering critical cell proliferation and cell cycle-related regulators, cyclin D1, and cyclin-dependent kinase inhibitor p21, which negatively (p21) and positively (cyclin D1) regulate cell proliferation and cell cycle progression [34]. The cyclin-dependent kinase inhibitor p21 and cyclin D1 are the two miRNA-20a targeted proteins that negatively (p21) and positively (cyclin D1) regulate and control cell cycle progression and proliferation. In general, elevated expression of miRNA-20a induces a marked promotion in MM cell proliferation and a decrease in apoptosis, and vice versa [35,36].

Alternatively, dysregulated expression of miRNAs can contribute to tumorigenesis by modulating tumour suppressor genes and oncogene signalling pathways. For example, critical components of key signalling pathways, such as Myc, p53, phosphatase and tensin homolog, and NF-κB are inhibited by miRNAs, leading to the description of miRNA functions as either oncogene or tumour suppressor genes. Deregulation of miRNA is not a random event in MM. Expression of miRNAs can be dysregulated due to different chromosomal aberrations and genetic mechanisms or could be regulated directly by proteins involved in the biosynthesis of miRNAs. These miRNAs in turn, regulate the expression of other genes that contribute to the progression and invasiveness of the disease [26]. Specific miRNAs, acting upstream or downstream of p53, or by repressing members of the B-cell Lymphoma protein (BCL) family, can result in successful apoptosis in normal cells. In contrast, miRNA epigenetic silencing could result in altered apoptosis rates. These miRNAs can indirectly promote the synthesis of vascular endothelial growth factor (VEGF) by targeting hypoxia-inducible factor (HIF-1) [15].

miRNA-21 has a significant role in the IL6/STAT-3 pathway which contributes to over-expression of MM compared to the controls. The up-regulation of miRNA-21 facilitates the activation of IL6 Janus Kinase (JAK) - STAT pathway. STAT-3 is a major mediator of growth, proliferation, and survival of myeloma cells conferred by BM microenvironment. The IL6/STAT-3 activation enhances myeloma cell survival through activation of anti-apoptotic protein Mcl-1 and Bcl-XL family. On the other hand, miRNA-20a is overexpressed in MM by inactivating an apoptotic gene, a negative regulator of IL-6/STAT-3 pathway [7].

The up-regulation of some miRNAs like miRNA-222, miRNA-218, miRNA-34a, miRNA-1274A, miRNA-138, miRNA-10b, and miRNA-1243 are shown to be involved in the proliferation of myeloma cells in MM patients [37]. On the contrary, over-expression of miRNA-191, miR-130a, let-7d, miRNA-103, let-7e, miRNA-744, miRNA-151-5p, miRNA-192 miRNA-215, miRNA-202, miRNA-126-3p, miRNA-140, miRNA-29b, and miRNA-15a/16 inhibit the growth and survival of MM cells [38-42].

Role of miRNAs in angiogenesis during MM

Angiogenesis is a means of supplying oxygen and nutrients to the growing number of tumor cells, which exists not only in solid tumours but also in hematological malignancies, such as MM [26]. Angiogenesis plays a crucial role in the pathogenesis and progression of MM. It greatly depends on the interactions of MM cells with the interaction of MM cells with the BMSCs, particularly endothelial cells and macrophages. These interactions may lead to changes in the expression of different array miRNAs and consequently changes the regulation of their target gene expression. A variety of chromosomal abnormalities such as translocations, gene mutations, and epigenetic alterations are involved in myelomagenesis. In addition to these oncogenic events, interactions between MM cells and the BM microenvironment are known to play a critical role in MM cell growth, survival, differentiation, migration, and chemotherapeutic resistance [43,44].

The tumour microenvironment, which comprises a variety of cell types, can secrete angiogenic cytokines including VEGF, platelet-derived growth factor and fibroblast growth factor thus promoting tumour angiogenesis via endothelial cell activation. Angiogenesis, a prominent feature of MM, can predict the prognosis of MM patients and is a hallmark of MM development and progression [45].

It is also a chemo-attractant to macrophages and a regulator of matrix metalloproteinase 9 (MMP-9); thus, it

can also indirectly enhance angiogenesis. Therefore, VEGF is perhaps the most potent pro-angiogenic factor known, and its enhanced expression, along with other pro-angiogenic factors, is regulated at several checkpoints including by miRNAs. First, hypoxia induces VEGF expression by the binding of the HIF-1α transcription factor to its binding site on the VEGF promoter, as well as to other pro-angiogenic factors like angiopoietin-2, MMPs, and semaphoring 4D. Local and chronic hypoxia generated in the BM due to the increased metabolic needs of proliferating MM cells. This prolonged hypoxic microenvironment exerts pressure on the malignant cells and those surviving MM cells, which become hypoxia-resistant to secrete twice the amount of miRNA-135b-containing exosomes. These exosomes were taken up by endothelial cells, and their cargo of miRNA-135b directly targeted the factor inhibiting HIF-1, which inhibits HIF-1α activity. Thus, prolonged, but not acute hypoxia, can mediate interactions between MM tumour cells and endothelial cells to elevate angiogenesis [26].

Potential role of miRNAs in MM diagnosis

Bone marrow biopsy is the gold standard for the clinical diagnosis of MM. However, it is a difficult and painful procedure. Therefore, there is a need to identify a more sensitive, convenient, and noninvasive biomarker to apply in the clinical diagnosis of MM. For this role, circulating serum miRNAs could have the potential to be the diagnostic markers for MM [3]. miRNAs circulate within the bloodstream in a stable and extracellular form, and thus are more readily available genetic material for relevant studies. Therefore, miRNAs represent readily accessible tools and an unprecedented means to diagnose diseases at an earlier stage and serve as a new class of powerful and minimally invasive biomarkers [20]. miRNAs hold great promise as readily accessible biomarkers to facilitate early disease detection of an asymptomatic stage - MGUS and to monitor the progression of MM [46,47].

Five miRNAs such as miRNA-744, miRNA-130a, let-7d, let-7e, and miRNA-34a have differentiated the stages (MM and MGUS). Combination of serum miRNA-34a and let-7e proved a more powerful discriminating tool between MGUS and MM [37]. Other evidence also reported that miRNA-720, miR-1308, and miRNA-1246 were found to have potential as diagnostic biomarkers for MM [9]. Moreover, additional meta-regression analyses showed that miRNA profiling was the sole source of heterogeneity, and the diagnostic accuracy of combined miRNAs was 6 times higher than a single one. Combined circulating miRNAs in serum or plasma may be a highly effective biomarker for the diagnosis of MM [48].

An additional, similar study also revealed that circulating serum miRNA-29a, miRNA-155, and miRNA-16 showed the potential to discriminate newly diagnosed MM patients from healthy donors. Serum miRNA-29a and miRNA-16 expression levels were found to be upregulated, while miRNA-155 was downregulated in MM patients. Meanwhile, serum miRNA-29a level in MM patients could be useful for the initiation of chemotherapy and for monitoring the disease status [19].

Recent studies argued that about 90% of the circulating miRNAs are potentially found in the plasma and serum. Serum/plasma miRNAs were co-fractionated with protein complexes rather than encapsulated by vesicles. Similarly, it is confirmed that the expression levels of miRNAs in serum and plasma are correlated, and both of them were suitable for detections of miRNAs as blood-based biomarkers. These results suggested that ethnicity did no influence the diagnostic accuracy of circulating miRNAs. Nevertheless, multiple miRNAs combined are superior diagnostic markers and can significantly differentiate the MM patients and healthy persons [48].

A panel of circulating miRNAs can considerably improve their diagnostic efficiency in MM. Another systematic review and meta-analysis conducted by Zhang also revealed that the combination of miRNA-34a and let-7e could differentiate MM cases from healthy donors with high diagnostic accuracy. Furthermore, miRNA-1308 and miRNA-720 together is an even more powerful diagnostic tool to distinguish normal healthy controls from MGUS and MM patients [48].

Role of microRNAs as a potential therapy for MM

miRNAs possess promising therapeutic potential in cancer because they can target many important genes or pathways at the same time. Several deregulated miRNAs have been identified and their important functions demonstrated. Attempts have been made to use miRNA expression profiles as biomarkers for MM progression, or classification of the MM cells into specific cytogenetic subtypes. Some miRNA arrays have been used to identify specific signatures or miRNA profiles that characterize different stages of MM progression and differentiate between MGUS and MM. Since miRNAs are involved in MM pathogenesis and regulate many of the molecular processes that dictate the course of the disease, it is reasonable to assume that miRNA profiling or determination of the expression of a specific miRNA may have diagnostic and/or prognostic value. Given their reported stability in serum, miRNA expression may represent novel non-invasive biomarkers of MM. This seems a promising direction for further study [26].

Furthermore, the dysregulated expression of miRNAs places them as a novel candidate therapeutic targets. Because miRNAs simultaneously target the expression of several genes and regulate key signalling pathways, targeting them is likely to be more beneficial than conventional approaches of targeting a single protein with a single drug. The problem of delivering small RNA molecules to tumour cells within the BM without using viral vectors and then making sure that the miRNAs or antagomirs are taken up specifically by the tumour cells has been addressed by developing lipid-based or poly-

mer-based delivery systems. Another possible advantage of using miRNA for therapy is the relative ease of detecting the aberrant expression of specific miRNAs in the serum of MM patients or even in their BM and the ability to closely follow up on changes of miRNAs expression in response to treatment. Collectively, in the future, these advantages may promote a personalized medicine approach, where patients will receive specifically-tailored antagonists or miRNA mimics according to their personal miRNA expression profile, hopefully increasing the success of the treatment [26].

There are two methods of the approach of miRNA-based therapy such as miRNA antagonists and miRNA mimics. Antagonists or anti-miRNAs are commercially synthetic RNAs that bind to complementary sequences of specific miRNA which inhibit endogenous target miRNAs [49].

miRNA mimics are used to restore a loss of function of miRNA. This approach, also known as 'miRNA replacement therapy', aims to re-introduce miRNAs into diseased cells that are normally expressed in healthy cells [49]. Evidence showed that transfection of pre-miRNA-15 and miRNA-16 used as a replacement of miRNA in MM cells. Cell cycle regulators (cyclin D1, D2) were suppressed by miRNA-15 and miRNA-16 transfection and mimic MM cells through inhibiting of AKT serine/threonine protein-kinase, ribosomal-protein-S6, MAP-kinases, and NF-κB-activator [50].

On the other hand, miRNA-125b is interferon regulatory factor 4 that triggers c-MYC promoting tumor growth and survival. Enforced expression of miRNA-125b induced apoptosis and autophagic related MM cell death. In addition to the above, miRNA-214 also suppresses MM cells and restoration promotes cell cycle arrest and apoptosis. The mechanism seems to be related to upregulation of p53, p21, and BAX via inhibition of the oncoprotein ankyrin. Anti-MM effects have been also associated with miRNA-137 and miRNA-19. The cytotoxic activity of these miRNAs has been mainly related to MCL1 targeting as for miRNA-29b. miRNA-202 has recently been described as downregulated in MM-related BMSCs and treatment with miRNA-202 mimics to restore its level within BMSCs overcame growth-promoting activity by reducing BCL-2. miRNA-based therapies can also modulate the BMM either directly by targeting MM cells without affecting the proliferation rate or by inducing an apoptosis [5].

CONCLUSION

The expression of miRNAs may be dysregulated due to complex cytogenetic complications in MM. These abnormal expressions of miRNAs play a major role in the pathogenesis, angiogenesis, and progression of MM. Serum/plasma miRNAs circulating within the human body in a stable form may play roles in the diagnosis, prognosis, and therapy of MM. These miRNAs will be helpful for the future by substituting the traditional chemotherapy/radiotherapy and will become a therapeutic target in MM. Only a limited number of studies have investigated the involvement of miRNA biomarkers for laboratory diagnosis as well as its therapeutic targets of MM. Therefore, further in-depth studies involving high-throughput functional assay techniques are required to identify potential miRNAs, which can be used as a diagnostic, prognostic, and therapeutic biomarker of MM.

Declaration:
We declare that this review paper is our original work.

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Not applicable.

Consent for Publication:
Not applicable.

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TT: Takes a major contributor in writing the manuscript. MM: Conceived the idea, reviewed and edited of the manuscript. Both authors read and approved the final manuscript.

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