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Role of microRNAs from monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma

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Abstract

microRNAs are increasingly recognized as significant players in oncogenesis and tumor biology through post-transcriptional gene regulation impacting broad pathways of proliferation, differentiation, apoptosis, metastasis and cell survival. Recent studies have found abnormal expression of microRNAs in multiple myeloma (MM). Currently, the precise role of these microRNAs in the biology of MM remains to be elucidated, although they are predicted to be involved in plasma cell proliferation, survival, homing, or in MM cell interactions with the bone marrow microenvironment. Furthermore, a limited number of studies focusing on MM precursor disease (monoclonal gammopathy of undetermined significance; MGUS) reveal significant differences in microRNA profiles between MGUS and normal plasma cells. Interestingly, several of the microRNAs differentially expressed in MGUS also show aberrant expression in MM suggesting a role in early myelomagenesis. MicroRNA profiles can discriminate molecular subtypes of MM that are defined based on gene expression profiling and cytogenetic abnormalities, demonstrating the potential diagnostic/prognostic utility of microRNA profiling for subclassification of MM. Given the relative stability of microRNA and ability to isolate microRNA from routine clinical specimens, microRNA analysis promises to facilitate personalized diagnostics and therapies, and to provide insights into the biology of early myelomagenesis.

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Introduction

MicroRNAs (miRNAs) are small non-coding RNAs involved in post-transcriptional gene regulation that have been demonstrated to play a role in regulation of apoptosis, proliferation, differentiation, cell survival and in oncogenesis¹. miRNAs are encoded throughout the genome and are transcribed by RNA polymerase II into a polyadenylated and capped primary or pri-miRNA which can be over 1 kb in length². The pri-miRNA is cleaved in the nucleus by the Drosha RNase III endonuclease, generating a hairpin precursor or pre-miRNA of ~60–100 nucleotides. Pre-miRNAs are transported to the cytoplasm and undergo further processing by Dicer, a ribonuclease III, which cleaves the pre-miRNA into an 18–24 nucleotide duplex (miRNA:miRNA*). Mature miRNAs form a complex with RISC (RNA-induced silencing complex) and the single stranded miRNA with specificity binds to the 3'UTR of target messenger RNAs (mRNAs) through complementary base pairing, leading to the degradation of the target mRNA transcript(s) or inhibition of translation². While the general effect of miRNAs is repression of translation, there is also evidence that some miRNAs can upregulate translation³.

Individual miRNAs can target multiple gene transcripts^{4,5} and can have a broad effect on gene expression⁶. It is estimated that 72–94% of gene transcripts are regulated by miRNAs⁵. Mature miRNAs are predominantly cytoplasmic and regulate translation, however, some miRNAs bearing a hexanucleotide motif AGUGUU including miR-29b, are found predominantly in the nucleus and are postulated to play a role in regulating gene transcription⁷. Over 1000 unique human miRNAs have been identified to date and are listed in the miRNA database (miRBase)⁸. Many more miRNAs are predicted to exist on the basis of bioinformatic analyses⁵. Up to one third of miRNAs in most cell types are thought to be currently unknown⁹.

Approximately one third of miRNAs are located within intronic regions of host genes and are cotranscribed with host genes under the same transcriptional regulatory mechanisms, and are processed from introns^{10,11}. Other miRNA genes are encoded as solitary genes with their own promoters¹. Additionally, several miRNA clusters have been identified such as the miR-17-92 cluster¹², which is critical for B-cell development¹³. The miR-17-92 cluster is transcribed as a single polycistronic primary transcript that is subsequently cleaved into seven individual separate mature miRNAs (miR-17-5p, miR-17-3p, miR-19a, miR-19b, miR-20a, miR-20a*, miR-92a) that repress target genes.

miRNA profiling has shown utility in the classification of solid tumors^{14,15} and diffuse large B-cell lymphoma⁹. When compared to normal cells of the same lineage, many cancers are reported to show significant downregulation or absence of key miRNAs¹⁴. In contrast, several studies indicate that MM and other B-cell malignancies maintain expression of lineage specific miRNAs¹⁶. Indeed, miRNAs show increased expression in MM^{17,18}. miRNAs that are deleted or downregulated in malignancy are predicted to target transcripts of oncogenes; likewise miRNAs that are increased may contribute to tumor biology by targeting tumor suppressor transcripts. Despite the limited number of miRNA studies in MM and its precursor state, MGUS, there is emerging evidence that specific miRNAs may play critical roles in normal plasma cell development and in early myelomagenesis.

miRNAs in normal B-cell and plasma cell differentiation

miRNA and mRNA profiling studies show that there are distinct miRNA profiles that correlate with specific stages of B-cell maturation^{19, 16}. The majority of differentially expressed miRNAs are predicted to target transcription factors involved in the orchestration of B-cell maturation. Jima et. al.⁹ used high throughput deep sequencing of normal B-cells and B-cell tumors to elucidate the B-cell small RNA transcriptome of normal B-cells and B-cell tumors. Through this process they identified 333 known miRNAs and 286 novel unknown candidate miRNAs⁹. Interestingly, 101 cases of diffuse large B-cell lymphoma were profiled and demonstrated that 25 miRNAs were equally efficacious in differentiating subgroups of diffuse large B-cell lymphoma as gene expression profiling (GEP). 6 of the 25 miRNAs were novel, underscoring the important roles undefined miRNAs may have in oncogenesis and tumor biology.

In a recent study by Zhang et. al.¹⁶, normal tonsils were disaggregated and sorted into naïve B-cells (CD19+/IgD+/CD27-/CD38+), germinal center (GC) B-cells (CD19+/IgD-/CD38++), memory B-cells (CD19+/IgD-/CD27+/CD38 dim+), and plasma cells (PC) (CD19dim+/IgD-/CD27++/CD38+++). The GC to PC transition showed differential expression of up to 33 miRNAs, the majority of which were more highly expressed in GC cells and were downregulated in PCs. Three specific miRNAs, miR-9, miR-30b and miR-30d were all downregulated in PCs and were shown to target the 3'UTR of the PRDM1 (Blimp1) transcript¹⁶. PRDM1 plays an essential role in normal plasmacytic differentiation²⁰ and evidence suggests that the downregulation of these specific miRNAs in the transition from GC to PC allows emergence of Blimp1 protein expression and permits plasma cell differentiation.

miRNA-181a and b are also important in B-cell differentiation¹⁹. Studies showed miRNA-181 is preferentially expressed in B-lymphoid lineage. When miRNA-181 was ectopically expressed in hematopoietic stem cells the fraction of B-cells was increased in both *in vitro* assays and *in vivo* in adult mice¹⁹. miRNA-142 is also preferentially expressed in myeloid and B-lineage cells and is postulated to play a role in early B-cell development¹⁹. Ventura et al.¹³ demonstrated that the miRNA-17~92 cluster is essential for B-cell development. Absence of miRNA-17~92 resulted in increased levels of the proapoptotic protein Bim and inhibited B-cell development at the pro-B to pre-B transition¹³. Additional validated targets of miRNA-17~92 include PTEN²¹ and E2F1²².

miRNAs in MGUS

Pichiorri et. al.¹⁸ in a seminal study, profiled the expression of 345 human miRNAs using a hybridization based custom miRNA array platform in CD138+ selected plasma cells from six MGUS patients, 16 MM patients, six normal controls, and 49 MM-derived cell lines. They found 41 miRNAs that were significantly (greater than 2 fold change with p-value < 0.01) upregulated in MGUS plasma cells relative to normal plasma cells and seven miRNAs that were significantly down regulated. The miRNAs that were increased in MGUS included miRNA-21, -181a, -93, the -106b~25 cluster, -25, and -106a (see Table). miRNA-328 was significantly decreased in MGUS. Of these miRNAs, miRA-21, -181a and the -106b~25

cluster were also upregulated in MM plasma cells. The findings suggested that these differentially expressed miRNAs play a role in early changes associated with development of the abnormal clonal plasma cell population in MGUS. Functional studies demonstrated that miRNA-181a and b, miRNA-106b~25, and miRNA-32 target the 3'UTR of p300-CBP-associated factor (PCAF) *in vitro* in MM cell lines. PCAF is a histone acetyl transferase that acetylates p53 and positively regulates p53. Antisense oligos inhibiting miRNA-181a and b, doubled the protein levels of PCAF and p53 suggesting the potential role these miRNAs may play in altering the p53 pathway in MGUS¹⁸. Studies in gastric cancer have shown that the miRNA-106b~25 cluster is activated by E2F1 and in turn regulates E2F1 in a negative feedback loop; in addition, this cluster impairs the TGF-beta tumor suppressor pathway, and targets the proapoptotic regulator BIM and the cell cycle inhibitor p21^{waf1/cip1},²³.

miRNA-21 is a putative oncogenic microRNA that is reported to be over-expressed in several cancers^{15, 24}. There is evidence that miRNA-21 may confer proliferative advantage to monoclonal plasma cells in MGUS and MM. Functional studies reported by Loffler et al.²⁵ demonstrated that IL-6 regulates miRNA-21 transcription in a STAT-3 dependent manner in MM cell lines. STAT3 binding sites were identified on an upstream enhancer of the miRNA-21 gene. Ectopic expression of miRNA-21 sustained growth and inhibited apoptosis in the absence of IL-6 in IL-6 dependent MM cells. Interestingly, others have shown that the transcription factor FoxO3a initiates apoptosis by transcriptionally repressing expression of mir-21²⁶.

miRNAs in malignant transformation of MGUS to MM

Pichiorri et. al.¹⁸ found miRNA-32 and the miRNA-17-92 cluster, including miRNA-19a and 19b, were significantly upregulated in MM and not in MGUS implicating a potential role for these miRNAs in malignant transformation of MGUS to MM¹⁸. miRNA-19a and 19b was increased > 100 fold in MM samples and >2000 fold in MM cell lines as compared to normal plasma cells and to MGUS. *In vitro* studies in MM cell lines, demonstrated that miRNA-19a and -19b down regulate SOCS1, which inhibits IL-6 signaling through JAK/STAT-3 pathway leading to constitutive activation of JAK/STAT-3 signaling in MM. Antisense oligos against miRNA-19a and -19b significantly increased SOCS-1 protein levels. Consistent with prior studies¹³, the miRNA-17~92 cluster was shown to target the proapoptotic regulator BIM in MM cell lines, conferring enhanced survival. Ectopic repression of miRNA-19 and miRNA-181a and -181b led to significant suppression of tumor growth *in vivo* in mice¹⁸. Other groups have demonstrated PTEN and E2F1^{22, 21} as targets of miRNA17~92 cluster with evidence that this cluster is regulated by c-Myc²². Interestingly, the therapeutic histone deacetylase inhibitor ITF2357, which has potent cytotoxicity to MM cell lines *in vitro*, was shown to significantly decrease expression of miR-19a and miR-19b in MM cell lines²⁷.

miRNAs in MM

In addition to the study by Pichiorri et. al., several other groups have found abnormal expression of microRNAs in MM. Roccaro et. al.²⁸ profiled the expression of 318 miRNAs using a liquid phase microbead Luminex assay from CD138+ selected plasma cells from 15

relapsed/refractory MM patients and 4 healthy donors, in addition to MM cell lines. miRNA-15a and -16 expression was significantly decreased in MM samples, validated by quantitative real time PCR. miRNA-15a and -16 are located on chromosome 13q14. These miRNAs are intronic and coexpressed within their host gene DLEU2 which is a putative tumor suppressor gene regulated by Myc²⁹. The 13q14 region is deleted in over 50% of MM³⁰ and also deleted in chronic lymphocytic leukemia⁶. All of the patients in the study with chromosome 13 deletions showed absence of miRNA-15a and -16; however even those without chromosome 13 deletions showed significantly decreased expression of miRNA-15a and -16 in this study. miRNA-15a and -16 were previously shown to target Bcl-2 in CLL³¹. Functional studies performed by Roccaro et. al. in MM cell lines demonstrated that miRNA-15a and -16 additionally regulate proliferation *in vitro* and *in vivo* by inhibiting AKT3, ribosomal-protein S6, cyclin D1, cyclin D2, and MAP-kinases. miRNA-15a and -16 inhibited TNF-alpha activation of the NF-kB-activator MAP3KIP3, and inhibited activation of p65, p50 and p52. *In vitro* and *in vivo* assays analyzing the bone marrow microenvironment in MM revealed that VEGF secretion by MM cells and angiogenesis in bone marrow stromal cells was inhibited by miRNA-15a and -16 as was the adhesion and migration of MM cells. When injected into mice, the homing of MM cells to the bone marrow was inhibited by miRNA-15a and -16. miRNAs that were statistically significantly increased in relapsed/refractory MM included miRNA-222, -221, -382 and -181a and -181b.

In contrast to the study by Roccaro et al., in a study of 26 newly diagnosed MM patients, Corthals et al.³² found miRNA-15a and -16 expression levels were often elevated in MM in comparison to normal plasma cells independent of the presence or absence of chromosome 13 deletions. miRNA profiling studies of newly diagnosed MM reported in Picchiori et al.¹⁸ and Zhou et al.¹⁷ also found miRNA-15a to be more highly expressed in newly diagnosed MM than in normal plasma cells. Taken together the findings suggest that miRNA-15a and -16 may be more important in advanced MM than in primary tumors. In primary tumors with a chromosome 13 deletion, miRNA-15a and -16 levels are likely maintained due to compensation of the non-deleted allele.

In a recent study by Unno et al.³³, plasma cells were profiled from two MM patients, two healthy controls, and MM cell lines using the Locked Nucleic Acid (LNA) microarray system containing sequences from 757 human miRs. They found 22 miRNAs that were upregulated in MM and 6 miRNAs that were downregulated. The findings were further confirmed through quantitative PCR on 10 additional MM patient samples. Of primary interest, four miRNA clusters were identified as significantly upregulated in MM, three of which were previously reported in Pichiorri et al. (miRNA-17-92; miRNA-106b-25; and miRNA-106a-92) and one (the miRNA-193b~365 cluster) was a novel finding. The miRNA-193b~365 cluster is highly conserved among vertebrates; however the function in MM remains to be elucidated.

Ronchetti et. al.³⁴ identified three intronic miRNAs, miRNA-335, miRNA-342, and miRNA-561, which showed coordinated expression with their host genes (MEST, EVL, and GULP1) in MM cell lines, and in a fraction MM primary tumor cells which were not correlated with known molecular subtypes. The precise role of these miRNAs in the biology

of MM is unclear, although they are predicted to be involved in plasma cell homing or in MM cell interactions with the bone marrow microenvironment³⁴.

Correlation of miRNA profiles with gene expression profiles and molecular subtypes of MM

The use of combined miRNA and mRNA profiling is a useful strategy for elucidating functional miRNA/mRNA regulatory networks, in addition to evaluating the utility of miRNA profiling to identify clinically relevant molecular subtypes of MM. In a recently published study by Zhou et al.¹⁷, purified CD138+ plasma cells from 52 newly diagnosed MM patients and two healthy donors were profiled using microarrays with sequences from 464 human miRNAs. GEP was also performed for the same samples. 95 of the miRNAs were expressed in MM cells with the remainder being absent or expressed at low levels. The total miRNA expression levels were higher in MM cells than in normal plasma cells. Forty miRNAs were differentially expressed in MM and 39 of these were expressed at significantly higher levels in MM than in normal plasma cells. Only one miR was expressed at lower levels in MM. Of note, this group previously defined a molecular subclassification of MM with prognostic risk scores and proliferation indexes for MM based on the expression levels of 70 genes^{35,36}. In their recent miRNA study, Zhou et al. found that global miRNA expression levels were correlated with previously validated mRNA-based risk stratification scores, proliferation indices, and high risk cancer gene sets. Interestingly, and in contrast to GEPs, all tested miRNAs were significantly upregulated in high risk MM defined by the 70-gene mRNA analysis model. The findings suggested that high expression levels of total miRNAs confer a poor prognosis in MM. Four of the miRNAs that were increased in MM and associated with a significant risk score (miRNA106a, -106b, -17p and -20b) were experimentally shown to target p21^{Waf1/Cip1} (CDKN1A), a reported tumor suppressor gene in MM^{37, 38, 39}. AGO2, a master regulator of miRNA genesis, was previously reported by this group to be a marker of disease prognosis. *In vitro* studies showed down regulation of miRNAs in MM cell lines upon silencing of AGO2, suggesting that AGO2 may play a role in increased global expression of miRNAs in MM¹⁷.

The correlation between miRNA profiles, transcriptional profiles, and recurrent cytogenetic/molecular abnormalities was also studied by Lionetti et al.⁴⁰ in 40 MM patients representing five translocation/cyclin (TC) groups⁴¹ and three healthy controls. miRNA arrays in this study contained sequences for 723 human miRNAs. Differential miRNA profiles were most prominently associated with IGH translocations. 26 miRs identified showed highly differential expression across the 5 TC groups. TC5 (patients with t(14;16) or t(14;20)) showed increased expression of miRNA-150, -133b, -99a, -133a, -155, -125b, -let7c, -1 -155*, and -34b. TC4 (patients with t(4;14)) showed increased expression of miRNA-125a-5p, let-7e, -99b, -222, -221, -221*, and -365. TC2 (including patients with low to moderate levels of the CCND1 gene and absence of an IGH translocation) showed increased expression of miRNA-874, -1237, -512-3p, -940, -933 and -1226*. TC1 (patients with t(11;14) or t(6;14)) showed increased expression of miRNA-361-3p, -582-5p, and -30e*. Several of the miRNAs that were differentially expressed mapped to chromosomal regions that frequently show deletions or gains in MM. miRNA-17 and -20a mapped to a

cluster at 13q31, which was deleted in 40% of the patients in this study. miRNA-1231, -205, and -215 map to the long arm of chromosome 1 which showed a gain in over 30% of the MM in this study. Loss of heterozygosity (LOH) was also associated with altered miRNA expression; there was a significant association between the LOH at 16q22.1-q23.1 and the expression of miR-140-3p. LOH on 16q has been reported to confer overall worse survival in MM ⁴².

Through integrative analysis of miRNA/mRNA expression profiles, Lionnetti et al. reconstructed a network model of functional interactions based on anticorrelated expression profiles. They identified a total of 23,729 regulatory relationships involving 628 miRNAs and 6435 predicted target genes. The number of target genes per miRNA ranged from 1 to 440, with an average of 34. Of the 26 miRNAs that were able to discriminate subgroups of MM in Lionnetti et al., miRNA-155, -221, -222, and -let-7 are the most well-described miRNAs. miRNA-155 shows increased expression in multiple B-cell malignancies ^{43, 44, 45} and is suggested to cooperate with MYC driving unregulated proliferation and impacting multiple signaling cascades including MAP kinases, PI3Kinase/Akt, and NFkB pathways ^{46, 45}. miRNA-221 and -222 putatively target C-KIT, p27, and p57 genes ^{47, 48}. The let-7 family is known to target RAS genes ⁴⁹ and oncogenes involved in the cell cycle progression ⁵⁰.

A tandem miRNA and mRNA profiling study was also performed by Gutierrez et al. ⁵¹ with 60 newly diagnosed MM selected to adequately represent recognized molecular subtypes of MM, and 5 healthy controls. Using a qRT-PCR based platform, profiles of 365 miRNAs were generated. When comparing miRNA profiles of the 60 MM samples vs. the normal plasma cells, they found 11 miRNAs that were significantly downregulated in MM: miRNA-375, -650, -214, -135b, -196a, -155, -203, -95, -486, -10a, and -196b. These findings are discordant with the findings of Picchori et al. and others who identified miRNA profiles with significantly increased miRNA expression in MM compared to normal PCs. Similar to the study by Lionnetti et al., this group found distinct miRNA signatures associated with specific molecular subgroups of MM (e.g., upregulation of the cluster miRNA-1/miRNA-133a in t(14;16) MM), although the specific miRNA profiles varied between the two studies.

Summary and conclusions

The results obtained in the studies reviewed had many overlapping findings underscoring the critical role specific miRNAs play in biology of MGUS and MM. There were some significant discrepancies which may be due to the different experimental platforms used for miRNA profiling, different numbers and/or subsets of miRNA sequences analyzed, different sample sizes for MM and controls, and/or different normalization or statistical methods used to analyze data. Ongoing validation and functional studies will be critical to further elucidate and define the roles of many miRNAs in MGUS and MM, particularly those that are newly discovered or relatively unknown.

GEP has been useful for the identification of subtypes of MM with important clinical implications for prognosis and response to therapy. The translation of GEPs into diagnostic

and clinical use is hindered by the need for fresh or frozen tissue for adequate isolation of mRNAs. In contrast, miRNAs are relatively stable and have been successfully isolated and amplified by quantitative RT-PCR from tissues processed in routine formalin-fixed paraffin-embedded methods^{52, 53}. miRNA may serve as superior biologic material for molecular classification in normal clinical laboratory settings. Studies in solid tumors demonstrate the utility of miRNA profiling in classification of tumors^{14, 9, 15}. Differential miRNA profiles have already been shown to accurately differentiate molecular subgroups of other hematologic malignancies including AML⁵⁴, CLL⁵⁵, and diffuse large B-cell lymphoma¹⁶. Several studies summarized in this review indicate that miRNA profiling may be as robust as GEPs in subclassification of MM into recognized clinically relevant molecular groups. Additionally, miRNAs are stable in peripheral blood and may be ideally suited as biomarkers for disease diagnosis, prognosis, or monitoring response to therapy. The role of miRNA in early myelomagenesis (MGUS) is emerging based on limited studies to date and promises to quickly unfold in this rapidly evolving field.

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Table

Selected miRNAs differentially expressed in MGUS and/or MM

miRNA	Finding in MGUS and/or MM	Predicted Targets or Function
1	Increased expression in t(14;16) MM ^{40,51}	Unclear
15a and 16	Decreased in relapsed/refractory MM ²⁸ ; not in primary MM ^{32,17,18} or in MGUS ¹⁸	Bcl-2 ³¹ ; AKT3, ribosomal-protein S6, cyclinD1 and D2, MAP-kinases ²⁸ ; inhibits proliferation ²⁹
21	Increased in MGUS ¹⁸ and MM ^{18,17}	Regulated by IL-6 in a STAT3 dependent mech, promotes proliferation and inhibits apoptosis ²⁵ ; negatively regulated by FoxO3a ²⁶
17~92 cluster: miR-17-5p, miR-17-3p, miR-19a, miR-19b, miR-20a, miR-20a*, miR-92a	Increased in MM ^{18,33} not in MGUS ¹⁸	Essential for B-cell development ¹³ , targets proapoptotic BIM ¹³ , PTEN and E2F1 ^{21,22} ; reportedly regulated by MYC ²²
17-5p	Increased in MM ¹⁷	Targets CDKN1/P21WAF1/CIP1 ¹⁷
19a and 19b	Increased in MM ¹⁸ not in MGUS ¹⁸	Down regulates SOCS1 which inhibits IL-6 signalling ¹⁸ ; suppresses tumor growth in mice ¹⁸
20b	Increased in MM ¹⁷	Targets CDKN1/P21WAF1/CIP1 ¹⁷
32	Increased in MM ¹⁸ not in MGUS ¹⁸	Targets p300-CBP-associated factor (PCAF) involved in p53 regulation ¹⁸
99b	Increased in t(4;14) MM ⁴⁰	Unclear
106b~25 cluster	Increased in MGUS ¹⁸ and MM ¹⁸	Targets p300-CBP-associated factor involved in p53 regulation ¹⁸ ; Targets BIM, p21, and impairs the TGF-beta tumor suppressor pathway ²³
106a, 106b	Increased in MM ¹⁷	Targets CDKN1/P21WAF1/CIP1 ¹⁷
125a-5p	Increased in t(4;14) MM ⁴⁰	Unclear
155	Increased in t(14;16)t(14;20) MM ⁴⁰ ; decreased in MM versus normal PCs ⁵¹	Increased in multiple B-cell malignancies ^{43,44,45} ; cooperates with MYC impacting MAPKineses, PI3/AKT, and NFkB pathways ^{47,48}
181a and/or b	Increased in MGUS ¹⁸ and MM ^{18,28,17}	Targets p300-CBP-associated factor involved in p53 regulation ¹⁸ ; suppresses tumor growth in mice ¹⁸ ; regulates B and T-cell differentiation ¹⁹
193b-365 cluster	Increased in MM ³³	Unclear
221 and 222	Increased in MGUS ¹⁸ and MM ^{18,28,40}	C-KIT, p27, p57 ^{47,48}
382	Increased in relapsed refractory MM ²⁸	Unclear
582-5p	Increased in t(11;14) MM ⁴⁰	Unclear
Let-7 family	Increased in t(4;14) MM ⁴⁰	Targets RAS genes ⁴⁹ and genes involved in the cell cycle (MYC, HMGA2, CDk6, CDC25) ⁵⁰

MGUS: monoclonal gammopathy of undetermined significance

MM: multiple myeloma