MicroRNAs: new players in multiple myeloma

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MicroRNAs (miRNAs) are short non-coding RNAs that play critical roles in numerous cellular processes through post-transcriptional regulating functions. The aberrant role of miRNAs has been reported in a number of hematopoietic malignancies including multiple myeloma (MM). In this review we summarize the current knowledge on roles of miRNAs in the pathogenesis of MM.

Keywords: miRNAs, multiple myeloma, microarray, profiling, p53, MDM2

INTRODUCTION

Multiple myeloma (MM) is a clonal B-cell malignancy characterized by the aberrant expansion of plasma cells (PCs) within the bone marrow, as well as into extramedullary sites (Bommert et al., 2006; Mahindra et al., 2010). The American Cancer Society estimates that 20,180 new cases of MM (11,170 in men and 9,010 in women) will be diagnosed while 10,650 deaths are expected to occur in the United States in 2010 (A.C.S., 2010). The lifetime risk of getting MM is 1 in 159 (0.63%) and the 5-year relative survival rate for MM is around 35%. Although the exact causes of MM remain elusive, our understanding of the cellular events underlying MM development is greatly advancing. This has been facilitated by extensive research that enabled thorough analysis of the changes during the pathogenesis of MM.

Multiple myeloma develops from a benign condition called monoclonal gammopathy of undetermined significance (MGUS) (Weiss et al., 2009). Individuals with MGUS often remain stable for years and do not require treatment. However, for unknown reasons, this benign condition can evolve into MM at a rate of ~1% per year, with some MMs developing after many years (Kuehl and Bergsagel, 2002; Fonseca et al., 2009). Malignant PCs progressively infiltrate the bone marrow and produce monoclonal immunoglobulin (Ig) (M protein) or an Ig chain (M-component). It is well known that the bone marrow microenvironment plays a prominent role in the biology of MM; adhesion of MM cells to the bone marrow stroma triggers cytokine production, enhances cell proliferation and resistance to chemotherapy by activation of NFκB, PI3K/AKT, and STAT-3 pathways through IL-6 (Kastrinakis et al., 2000; Katz, 2010). The

progression of MM is associated with osteolytic lesions, bone marrow failure, suppression of normal Ig production and renal insufficiency.

Multiple myeloma is characterized by various cytogenetic abnormalities some of which are of prognostic significance (Fonseca et al., 2004; Avet-Loiseau et al., 2007). Specifically, del(13), t(11;14), t(4;14), t(14;16), hyperdiploidy, MYC translocations, and del(17p) are among the most common cytogenetic changes in MM patients. Univariate statistical analyses revealed that del(13), t(4;14), non-hyperdiploidy, and del(17p) negatively impact both the event-free survival and the overall survival, whereas t(11;14) and MYC translocations are thought not to influence the prognosis. Nevertheless, cumulative evidence suggest that only t(4;14) and del(17p) retained prognostic value for both the event-free and overall survivals as assessed by multivariate analyses (Avet-Loiseau et al., 2007; Neben et al., 2010). These results have implications for risk-adapted management for patients with MM and may suggest that different treatment options might have variable success, depending on the underlying genetic nature of the clone. Despite these recent advances in cytogenetics, oncogenomics, and MM cell–stroma interactions (Avet-Loiseau et al., 2007; Katz, 2010; Neben et al., 2010; Anderson, 2011), further studies are required to better reveal critical players in MM development. The emerging role of microRNAs (miRNAs) in numerous types of human cancer led us and others to hypothesize that this group of non-coding genes might also be involved in the pathogenesis of MM.

MicroRNAs are a relatively recently identified class of regulatory non-coding RNAs, typically 19–25 nucleotides in length, that function primarily by targeting specific messenger RNAs (mRNAs)

for degradation or inhibition of translation through base pairing to partially or fully complementary sites (Ambros, 2003; Bartel, 2004). MiRNAs are involved in a variety of biological processes, including development, differentiation, apoptosis, survival, senescence, and metabolism (Ambros, 2004; He and Hannon, 2004). Several hundred miRNAs have been identified and studied so far, but it is predicted that there are many more miRNAs whose significance and role in homeostasis and pathology is yet to be identified. In 2002, *miR-15a* and *miR-16-1* located at 13q14.3, a region that is commonly deleted in chronic lymphocytic leukemia (CLL) and MM, were identified as potential cancer genes in the pathogenesis of CLL (Calin et al., 2002). This finding provided the first evidence that miRNA genes might be important for cancer development. Later on, the use of different approaches including microarrays (Calin et al., 2004; Volinia et al., 2006) and quantitative reverse transcription PCR (qRT-PCR; Schmittgen et al., 2004; Jiang et al., 2005), revealed that miRNAs are differentially expressed in cancer cells and identified significant miRNA genes that are of biological and clinical relevance in human diseases (Volinia et al., 2006; Liu et al., 2008). Several miRNAs have been identified as oncomirs (miRNAs that are amplified or overexpressed in cancer and were shown to have a promoting role in the development of primary tumors) or tumor suppressors (miRNAs that are deleted or reduced in cancer cells and their loss is associated with tumor development; reviewed in Calin and Croce, 2006; Ventura and Jacks, 2009). Various causes of deregulated miRNA expression in cancer have been identified including changes in gene copy number (CN), chromosomal translocation, mutations, transcriptional activation, epigenetic silencing and defective miRNA processing, and biogenesis (Calin and Croce, 2006). Collectively, it is realized that deregulation of, at least some, miRNAs might also contribute to tumorigenesis.

During the last 4 years, several studies investigated miRNAs expression and function in the pathogenesis of MM. In this review, we will discuss the classification of these miRNAs, their cause of deregulation, the mechanisms by which these genes exert their functions and the clinical relevance of their expression in MM.

MiRNAs ARE DIFFERENTIALLY EXPRESSED IN MM

Initially, it has been shown that *miR-21* levels in MM cells are controlled in a STAT-3-IL-6 dependent manner (Loffler et al., 2007). Treatment of IL-6-dependent MM cells with IL-6 activated STAT-3, which in turn enhanced *miR-21* transcription. Importantly, ectopic expression of *miR-21* was sufficient to sustain growth of IL-6-dependent MM cells in the absence of IL-6. Subsequent studies showed that *miR-21* levels are upregulated in MM and MGUS samples with respect to its levels in healthy PCs (Pichiorri et al., 2008; Zhou et al., 2010, see below). These results suggested that miRNAs levels might be implicated in MM pathogenesis.

In 2008, we reported a unique and comprehensive miRNA expression profiling of normal PCs, MGUS, and MM (Pichiorri et al., 2008). We utilized both a custom-made microarray chip and qRT-PCR of CD138+ normal PCs, MGUS, primary samples and MM cell lines. When compared with normal PCs, MGUS patients revealed a group of upregulated miRNAs with known oncogenic potential including *miR-21* and the *miR-106~25* cluster. Although we are aware that the small set of MGUS samples ($n = 10$) used for these analyses could limit its significance, our study for the first time suggested that miRNAs deregulation could be associated

with early stages of PC transformation. The fact that both *miR-21* and the *miR-106~25* cluster were shown to target, among others, *PTEN*, *BIM,* and *p21*, tumor suppressors that inhibit survival and promote apoptosis, could suggest that these miRNAs predispose to secondary events that might ultimately lead to full blown myeloma.

To better shed light on the role of miRNAs in MM, we next analyzed their expression in a group of MM cell lines and normal PCs (Pichiorri et al., 2008). Our data revealed a signature comprised of upregulated miRNAs, including among others *miR-32*, *miR-21*, miR-17~92, *miR-106~25*, and *miR-181a/b*. The Myc related *miR-17~92* cluster were highly expressed only in MM patients, suggesting that these miRNAs are MM-specific and their expression could be related to Myc upregulation during MM progression (Chesi et al., 2008). To further investigate the potential involvement of miRNAs in MM, Zhou et al. (2010) performed integrative analyses of both miRNA expression profiles and protein coding gene expression profiles of myeloma cells. Their analyses reveal a global increase in miRNA expression in high-risk (poor prognosis) MM patients. This association was reinforced by the increase in viability of MM cells depleted of Argonaute 2 (*AGO2*), a master regulator of miRNA maturation and function (Liu et al., 2004; Diederichs and Haber, 2007). In fact, AGO2 is also involved in B-cell differentiation (O'carroll et al., 2007) and was reported as an important marker for MM disease prognosis (Shaughnessy et al., 2007). Nevertheless, expression of AGO2 did not significantly associate with global miRNA (Zhou et al., 2010) suggesting that other factors might contribute to the overall deregulation of miRNA in MM. Analysis of miRNA expression by another group revealed upregulation of *miR-193b-365* among previously identified oncomiRs (Unno et al., 2009).

Intriguingly, many of the aforementioned miRNAs were previously identified (Pichiorri et al., 2008), though others were not or had an opposite trend. These differences could stem from the different platforms used for analyzing miRNA expression, number of cases analyzed and/or genetic and cytogenetic abnormalities in the different cases or cell lines. Furthermore, it is possible that miRNAs play opposing roles at different stages of the disease; i.e., in MGUS versus MM. Regardless of the disagreement among the different studies, the overall conclusions confirm that deregulation of miRNA expression adds a further level to our understanding of the biological and clinical variability of MM and warrants further investigations.

MECHANISMS OF miRNA DEREGULATION IN MM

Additional studies further demonstrated deregulation of miRNA expression in the different subtypes of MM and shed light on the mechanism of this alteration. Lionetti et al. employed an integrative high-resolution microarray analysis of miRNAs and DNA CN or gene expression profiles in MM cell lines (Lionetti et al., 2009). These analyses revealed the deregulation of 16 miRNAs mapped to chromosomal regions frequently involved in allelic imbalances in MM. Among these were *miR-22* at 17p13.3, *miR-106b* and *miR-25* at 7q22.1, *miR-15a* at 13q14.3, *miR-21* at 17q23.1, and *miR-92b* at 1q22. In another study, it was shown that expression of *miR-15a/16-1* cluster at 13q14 display a range of expression patterns in MM cases independent of chromosome 13 status (Roccaro et al., 2009). Further, 32 intragenic miRNAs significantly correlated

with that of their host genes of which some are known genes implicated in MM including *miR-152-COPZ2, miR-335-MEST,* and *miR-342-3p-EVL* (Ronchetti et al., 2008; Lionetti et al., 2009). Collectively, these data suggest that CN and frequent co-expression of intronic miRNAs with their respective host genes could be leading mechanisms to altered miRNAs expression in MM.

Since MM is characterized by very complex cytogenetic aberrations that affects prognosis and molecular profiling (Sawyer, 2011), it is likely that these aberrations might affect miRNA expression as well. Indeed, recent studies have shown, for example, that upregulation of *miR-1* and *miR-133a* is correlated with *t*(14;16) MM cases suggesting that deregulation of miRNA expression could be associated with chromosomal aberrations (Gutierrez et al., 2010). Other examples are shown in **Table 1**.

Impaired processing of miRNAs was also reported to be associated with high-risk MM. The findings of Zhou et al. (2010) clearly suggest that depletion of AGO2, involved in miRNA genesis and functionality, is associated with growth arrest and apoptosis in MM cells. Altered expression of AGO2 might lead to impaired miRNAs genesis thereby affecting their functions in MM pathogenesis. In agreement, it was recently shown that altered expression of Dicer, but not Drosha, might be associated with progression and prognosis of MM. In particular, it was suggested that MGUS premalignant cases display higher levels of Dicer than do SMM and MM cases and are very similar to normal PCs (Sarasquete et al., 2010). Moreover, higher expression of Dicer was associated with improved progression-free survival in symptomatic MM cases. Whether upregulation of Dicer in MGUS and MM is associated

Table 1 | miRNA genes deregulated in specific cytogenetic aberrations in multiple myeloma.

Cytogenetic Group	Deregulated miRNA	Chromosomal location	microRNA target
t (4;14)[a]	Hsa-miR-133b	6p12.2	FSCN1
	Hsa-miR-135b	1q32.1	
	Hsa -miR-146a	5q34	IRAK1, Fas, Smad4, TBP, CCL8/MCP-2
	Hsa -miR-155	21q21.3	KPC1, IL-13Rα1, CYR61, SMAD1, SMAD2SMAD5, HIVEP2, CEBPB, RUNX2, MYO10, PKIalpha, JARID2, AGTR1
	Hsa-miR-193a	17q11.2	
	Hsa-miR-196b	7p15.2	
	Hsa-miR-203	14q32.33	P63, SOCS-3
	Hsa-miR-215	1q41	DHFR, TS
	Hsa-miR-342	14q32.2	
	Hsa-miR-375	2q35	YAP, RASD1, PDK1, 14 3.3zeta
	Hsa-miR-650	22q11.22	NDRG2, ING4
t (11;14)[a]	Hsa-miR-95	4p16.1	SNX1
	Hsa-miR-125a	19q13.41	PDPN, BAK1, KLF13, preproET1, ARID3B, HuR, ERBB2, ERBB3
	Hsa-miR-184	15q25.1	AKT2
	Hsa-miR-199a	19p13.2/1q24.3	CD44, mTOR, c-MET, HIF-1alpha
	Hsa-miR-215	1q41	DHFR, TS
	Hsa-miR-375	2q35	YAP, RASD1, PDK1, 14 3.3zeta
	Hsa-miR-650	22q11.22	NDRG2, ING4
t (14;16)[b]	Hsa-miR-1	20q13.33	TAGLN2, KLF4, c-Met
	Hsa-miR-99b	19q13.41	
	Hsa-miR-125a-5p	19q13.41	PDPN, BAK1, KLF13, preproET1, ARID3B, HuR, ERBB2, ERBB3
	Hsa-miR-133a	18q11.2/20q13.33	
	Hsa-miR-135b	1q32.1	
	Hsa-miR-196b	7p15.2	
	Hsa-miR-214	1q24.3	Pten
	Hsa-miR-375	2q35	YAP, RASD1, PDK1, 14 3.3zeta
	Hsa-miR-642	19q13.32	
Del 13q14[c]	Hsa-miR-15a-16 cluster	13q14.3	E2F, CCND1, WNT3A, BCL2
	Hsa-miR-181a/b	1q32.1/9q33.3	RASSF1A, TIMP3, NLK, Prox1, HOX-A11
	Hsa-miR-221	Xp11.3	P27, Ets1, PUMA, p57, TIMP3, Bmf, PTEN, Mdm2
	Hsa-miR-222	Xp11.3	P27, PUMA, p57, TIMP3, PTEN
	Hsa-miR-382	14q32.31	SOD2, NPM1, PSPC1, HSPD1, ECH1

[a]*Gutierrez et al. (2010).*
[b]*Lionetti et al. (2009), Gutierrez et al. (2010).*
[c]*Roccaro et al. (2009), Corthals et al. (2010).*

with impaired miRNA expression and function is still to be determined. These findings may shed light on the potential prognostic and therapeutic strategies in which regulators for both miRNA maturation and function, such as AGO2 and Dicer, might be used as prognostic tools and/or treatments of MM.

DEREGULATION OF miRNAS REGULATES CRITICAL GENES ASSOCIATED WITH MM

Deregulation of miRNAs was shown to be associated with targeting coding genes that are implicated in MM. For example, *miR-17~92* cluster located at 13q31-32, a region frequently amplified in malignant B-cell lymphomas (He et al., 2005), and overexpressed in 65% of the B-cell lymphoma patients (Ota et al., 2004), has been shown to contribute to B-cell lymphoma by targeting *PTEN, E2F1,* and *BIM*. By overexpressing *miR-17~*92 in lymphoid progenitor cells from mice that carry the *MYC* transgene, He et al. reported an accelerated lymphomagenesis by the cluster (He et al., 2005). A different group generated mice with high *miR-17-92* lymphocytic expression and observed a higher rate of lymphoproliferative disorders, autoimmunity, and premature death in the transgenic mice (Xiao et al., 2008). Further, the work of Ventura et al. (2008) demonstrated that this cluster is essential for B-cell development and that its deficiency leads to increased levels of the pro-apoptotic protein BIM thereby inhibiting the transition of pro-B to pre-B stage. Therefore, it is likely that upregulation of the *miR-17~92* cluster negatively regulates these critical tumor suppressor genes contributing to PCs transformation and MM progression.

On the other hand, the *miR-106~25* cluster together with *miR-32* were shown to target *PCAF* (Pichiorri et al., 2008), a p53 positive regulator (Schiltz and Nakatani, 2000; Linares et al., 2007). Downregulation of PCAF could keep p53 at low level via its histone acetyltransferase function contributing to MM progression. Additionally, *miR-106a/b, miR-17-5,* and *miR-20b* were shown to target the *CDKN1A1*/p21 cell cycle regulator implicated in MM (Chen et al., 1999; Lavelle et al., 2001). Another central pathway that is deregulated in MM pathogenesis is the STAT-3/IL-6 pathway (Bommert et al., 2006). Targeting of this pathway by *miR-19a/b* was demonstrated in MM cells (Pichiorri et al., 2008). *MiR-19a/b* directly targets a negative regulator of IL-6, SOCS1, contributing to its frequent downregulation in MM cells. Importantly, xenograft studies using human MM cell lines treated with *miR-19a/b* and *miR-181a/b* antagonists (antimiRs) resulted in significant suppression of tumor growth in immunocompromised mice (Pichiorri et al., 2008).

MiRNAs 15a/16-1 FUNCTION AS TUMOR SUPPRESSORS IN MM

As mentioned above, *miR-15a* and *miR-16-1* are located at 13q14.3, a region that is commonly deleted in CLL and MM among other malignancies (Aqeilan et al., 2010). Deletion of this region occurs in more than 50% of cases and is believed to take place at an early stage of the disease suggesting that it might be associated with MM pathogenesis (Fonseca et al., 2004). MiRNA expression profiling of bone marrow derived CD138 + MM cells versus their normal cellular counterparts identified an MM-specific miRNA signature characterized by significant downregulation of *miR-15a/16* (Roccaro et al., 2009; Corthals et al., 2010). Similar to CLL, it was hypothesized that minimal deletion in 13q14.3 containing

the *miR-15a/16* cluster might have an oncosuppressor effect on MM pathogenesis. Indeed, the functional role of *miR-15a/16* in MM cells revealed their regulation of cell proliferation, survival, and cell cycle progression *in vitro* and *in vivo* (Roccaro et al., 2009). Recent studies from our laboratory have shown that the expression of the *DLEU-2* gene, located at 13q14.3 and containing *miR-15a/16* clusters, is completely absent in MM cells as well as in normal PCs. Nevertheless, *miR-15a* and *miR-16* are indeed downregulated. This might strongly suggest that the expression of these miRNAs could be driven from their related cluster at chromosome 3 (q25.33). Alternatively, an internal promoter unrelated to the host gene could drive their expression in PCs.

Although at the genetic level it is still considered an open question, at the molecular level *miR-15a/16* are shown to regulate cell cycle proteins including cyclinD1, cyclinD2, and CDC25A expression. Moreover, *miR-15a/16* target BCL2 expression (Aqeilan et al., 2010) and their overexpression in MM cells induces apoptosis as indicated by an increased percentage of sub-G1 population. *MiR-15a/16* were also shown to inhibit AKT3 and NFκB pathways in MM cells (Roccaro et al., 2009). Moreover, ectopic expression of *miR-15a/16* displayed antiangiogenic activity through regulation of VEGF. Most recently, Gatt et al. further investigated the tumor suppressor role of *miR-15a/16* in MM (Gatt et al. 2010). Using "sponge" lentiviral vectors directed toward the mature form of *miR-15a/16*, it was shown that inhibition of *miR-16* enhanced proliferative and invasive ability of MMS1 cells *in vitro*. Further, depletion of *miR-16* decreased animal survival in a xenograft model of MM through increasing tumor load and host angiogenesis. Gene expression profiling of *miR-16*-depleted MM1S cells revealed a large number of *miR-16* target genes including *FGFR1, VEGFa*, and *MDM4*. Importantly, *miR-16* levels correlated with 13q14 deletion in MM samples and with those having larger blood vessels (Gatt et al. 2010). These observations clearly support the fact that a single miRNA, for example *miR-16*, can have far-reaching effects on the overall function of the cell. Altogether, it is becoming obvious that *miR-15a/16* are critical regulators of MM pathogenesis through targeting of multiple pathways implicated in clonal PCs and bone marrow neoangiogenesis.

MiRNA FUNCTIONAL CROSS-TALK WITH P53 IN MULTIPLE MYELOMA

The tumor suppressor p53 is frequently inactivated by mutations or deletions in cancer. p53 acts as a potent transcription factor and can be activated in response to diverse stresses, leading to induction of cell cycle arrest, apoptosis, or senescence (Xue et al., 2007; Junttila and Evan, 2009). Although regulation of the p53 pathway is not fully understood at the molecular level, it has been well established that activated p53 suppresses cancer progression, underscoring why cancer cells have developed multiple mechanisms to disable p53 function (Danovi et al., 2004; Ventura et al., 2007). In human tumors that retain wild-type (WT) p53 (Lane, 2001; Junttila and Evan, 2009), p53 can be antagonized by murine double minute 2 (MDM2), a negative regulator of p53 that is overexpressed in many human tumors, offering a therapeutic strategy (Brown et al., 2009; Dickens et al., 2010). Indeed, it has been reported that inhibiting MDM2 expression can reactivate p53 in cancer cells, leading to their demise (Dickens et al., 2010; Saha et al., 2010a,b).

TP53 mutation is rarely detected at diagnosis in many hematological cancers including MM, CLL, acute myeloid leukemia, and Hodgkin's disease. Thus, numerous reports have shown that therapeutic induction of p53 might be particularly suitable for the treatment of hematological malignancies (Saha et al., 2010b) including MM (Kuehl and Bergsagel, 2002; Fonseca et al., 2009). Mutations of p53 are rare in untreated MM and the tumors appear to have intact, if perhaps suppressed, p53 function, suggesting that therapeutic modulation of the p53/MDM2 pathway holds promise to help the majority of patients. Several reports (Quesnel et al., 1994; Teoh et al., 1997) and our data suggest that MDM2 overexpression in MMs, not its gene amplification (Quesnel et al., 1994), could be responsible for p53 inactivation in cells retaining functional p53 pathways. This supports the idea that induction of p53 in this setting might be a suitable treatment for MM. Interestingly, in MM cells, expression of p53 protein levels can be rescued by antagonizing MDM2. Several reports have focused on the p53-mediated apoptotic pathway, upon endogenous p53 protein re-expression by the small-molecule MDM2 antagonists (Nutlins) and target genes which may be involved in p53-dependent apoptosis in MM cells which have been identified (Stuhmer and Bargou, 2006). We recently showed that alteration in miRNA expression during the progression from MGUS to newly diagnosed MM could partially be responsible for p53 inactivation in MM patients (Pichiorri et al., 2010). We found that, among others, *miR-192*, *194*, and *215*, which are downregulated in a subset of newly diagnosed MMs, can be transcriptionally activated by p53 and then modulate MDM2 expression (**Table 2**). Furthermore, ectopic re-expression of these miRNAs in MM cells increases the therapeutic action of MDM2 inhibitors *in vitro* and *in vivo* by enhancing their p53-activating effects. In addition, *miR-192* and *215* target the IGF pathway, preventing enhanced migration of PCs into bone marrow.

In seeking an explanation for the lack of expression of these miRNAs in MM, we have noted that the genes for these miRNAs are located in chromosomal regions in MM that are normally characterized by chromosome gain and translocations rather than deletions (Fonseca et al., 2009). This led us to hypothesize that other mechanisms are responsible for the observed downregulation of these miRNAs. Indeed, it turns out that epigenetic cross-talk between methylation and histone acetylation (Vaissiere et al., 2008; Walker et al., 2011) in the promoter of the *miR-194-2-*

192 is responsible for this silencing in MM cell lines. This could support the hypothesis that the transition from MGUS to MM could be favored by clonal selection of cells with the epigenetic inactivation not only of p53 but principally of its direct targets. This could be associated with a decreasing ability of p53 to down-modulate MDM2 expression thus tipping the regulatory balance in favor of MDM2 in MM cells. Whether methylation of the *miR-194-2-192* promoter in primary patients and in MGUS is responsible for this cluster inactivation is currently under investigation.
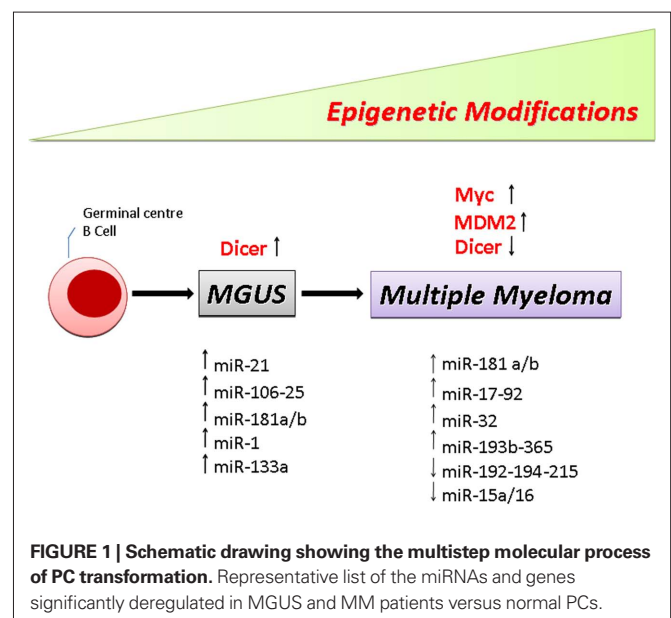
We and others also have shown that the upregulation of *miR-25* and *miR-181a* in MM cells of a specific set of miRNAs could negatively regulate the expression of the tumor suppressor gene p53 emphasizing the important cross-talk between p53 and aberrant microRNA expression in MM cells (Kumar et al., 2010; Pichiorri et al., 2010). Of note, monoallelic deletion of *TP53* in MM, which often seems to occur without mutation on the other allele, is associated with an extremely poor prognosis (Chng et al., 2007). This supports the idea that a decrease in *TP53* gene content is associated with tumor progression, which supports the hypothesis that a partial lack of expression of these miRNAs in MMs could create a p53 imbalance with direct biological consequences. The deletion of one copy of p53 by FISH has been uniformly found to be an adverse prognostic factor with all therapies used in the treatment of MM: alkylating agents, proteasome inhibitors, and immune modulator-based therapies (Lode et al., 2010). Due to the emerging role of epigenetic modifications in MM progression and the key role of p53 regulation by miRNAs it should be relevant to know if deacetylase inhibitors (DACi) could affect a set of miRNA expression in MM cells able to affect p53 re-expression making the cells more sensitive to further therapies.

CONCLUSIONS AND FUTURE PERSPECTIVES

Genetic alterations in MM have been identified and were shown to be associated with different biologic features and heterogeneity in clinical outcome. Recent research work highlights the possible contribution of a new class of non-coding genes, miRNA (**Figure 1**).

Table 2 | miRNAs deregulation associated with p53 expression in multiple myeloma.

p53 associated miRNAs	Chromosomal location	References
miR-34a	1p36.22	Chim et al. (2010)
miR-192	11q13.1	Pichiorri et al. (2010)
miR-215	11q13.1/1q41	Pichiorri et al. (2010)
miR-22	17p13.3	Lionetti et al. (2009)
miR-194	1q41	Pichiorri et al. (2010)
miR-125b	11q24.1/21q21.1	Kumar et al. (2010)
miR-25	7q22.1	Kumar et al. (2010)
miR-30d	8q24.22	Kumar et al. (2010)
miR-181-a/b	1q32.1/9q33.3	Pichiorri et al. (2008)

FIGURE 1 | Schematic drawing showing the multistep molecular process of PC transformation. Representative list of the miRNAs and genes significantly deregulated in MGUS and MM patients versus normal PCs.

Cumulative evidence indicates that miRNAs function as "fine" regulators of cell commitment and differentiation during human normal hematopoiesis (Fabbri et al., 2009). In parallel, several research groups are studying whether and how the miRNome differs in hematological malignancies (i.e., MM) with respect to the normal hematologic counterpart (i.e., CD138+ PCs). The study of miRNAs deregulation and their functional consequences in MM shed light on the molecular mechanisms of MM and B-cell malignancies in general. Intriguingly, the unique signature of miRNA gene expression in MM differentiates it from CD138+ B-cells and MGUS suggesting that miRNAs can be used in diagnosis and, probably, in prognosis and treatment of these tumors. Moreover, by revealing the molecular effectors of miRNAs, it is becoming evident that the use of miRNA and/or anti-miRNA molecules together with chemotherapeutic regimens might increase the success rate in treating MM. The interplay of miRNA genes and the bone marrow microenvironment and their role in MM pathogenesis and drug resistance is another interesting topic that would warrant investigation in the future.

Although for many years no single mouse model seemed capable of reproducing all facets of a specific human multiple myeloma, the Vk*MYC mice (Chesi et al., 2008) fulfill many of the biologic and genetic criteria of an ideal mouse model, showing a high degree of homology to the clinical phenotype of human MM. The Vk*MYC model relies upon a sporadic, yet precisely timed, physiologic mechanism for oncogenic activation, providing a model of tumorigenesis with high penetrance. It should be really interesting to study the miRNAs changes during myeloma development from MGUS to active MM in this mouse model. Whether MYC upregulation in early stages of the disease could lead to epigenetic modification in the genome that is related to p53 downregulation through MDM2 upregulation and higher chromosomal instability is another open question. While many answers are still pending, the need for further analysis of this new class of players in MM, and more general in cancer, is therefore required to better decipher miRNAs' role in this heterogeneous and incurable disease.

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