MicroRNAs Act as Decoy Molecules to Inhibit the Function of RNA Binding Proteins

Anna M. Eiring [*1]

Abstract

Altered microRNA (miRNA) expression contributes to aberrant post-transcriptional gene regulation in several types of cancers; however, their role in the progression of chronic myeloid leukemia (CML) from chronic phase (CML-CP) to blast crisis (CML-BC) is still largely unknown. To gain further insight into the role of miRNAs in CML disease progression, we used microarray-based techniques to analyze miRNA expression in CML-BC^{CD34+} compared to CML-CP^{CD34+} progenitors and in BCR/ABL-expressing myeloid cell lines compared to untransformed controls. Using this method, we identified a discrete number of miRNAs either upregulated (34 miRNAs) or downregulated (14 miRNAs) in both BCR/ABL^{+} cell lines and primary patient samples. Among the downregulated miRNAs, we focused our attention on miR-223 because of its reported role in myelopoiesis, miR-15a/16-1 because of their reported role as tumor suppressors, and miR-328, a miRNA with no currently known function. Northern blot and qRT-PCR analyses validated the results of our microarray analysis, revealing a marked reduction in miR-223, miR-328, miR-15a, and miR-16-1 expression in 32D-BCR/ABL and K562 cells (50-75% inhibition), and expression of these miRNAs was rescued upon treatment of cells with the tyrosine kinase inhibitor imatinib (2 mM; 24h).

Interestingly, sequence analysis of both miR-223 and miR-328 revealed homology with the hnRNP E2-mRNA binding site contained in the uORF spacer region of the *CEBPA* 5' UTR (*CEBPA* 5' uORF). hnRNP E2 is the RNA binding protein responsible for block of myeloid differentiation in CML-BC^{CD34+} progenitors, and does so by binding to the *CEBPA* 5' uORF to

[*]860 Biomedical Research Tower, 460 W. 12th Avenue, Columbus, OH, 43210, I would like to thank my advisor Dr. Danilo Perrotti for his insightful comments and Paolo Neviani for assistance with experimental procedures.

block its translation. Indeed, REMSA experiments revealed that synthetic miR-223 and, to a greater extent, miR-328, could bind efficiently to recombinant hnRNP E2 protein *in vitro* and compete for its binding to an oligoribonucleotide containing the *CEBPA* 5' uORF. Similarly, REMSA and UV-crosslinking showed that both miR-223 and miR-328 could bind endogenous hnRNP E2 from lysates of BCR/ABL-expressing but not parental cells, as well as from lysates of parental 32Dcl3 cells ectopically expressing a Flag-tagged hnRNP E2 protein. These results suggested that miR-223 and miR-328 may act as decoy molecules to interfere with the translation-regulatory activity of hnRNP E2. Consistent with this hypothesis, ectopic expression of miR-223 and, to a greater extent, miR-328, was capable of restoring G-CSF-driven granulocytic maturation of differentiation-arrested 32D-BCR/ABL cells and CML-BC^{CD34+} cells. Furthermore, C/EBPα protein levels were markedly increased in both miR-223- and miR-328-expressing 32D-BCR/ABL cells as well as in primary CML-BC^{CD34+} patient samples. Finally, while ectopic expression of miR-223, miR-328, miR-15a, or miR-16-1 had no significant effect on the viability of normal or BCR/ABL-expressing cells, ectopic expression of miR-15a/16-1 or miR-328 significantly impaired BCR/ABL-dependent colony forming ability (miR-15a/16-1: 65-75% inhibition, $P<0.001$; miR-328: 40-50% inhibition, $P<0.01$). Altogether, these data reinforce the importance of BCR/ABL-dependent post-transcriptional gene regulation during CML disease progression. Furthermore, they suggest a novel function for miRNAs as regulators of RNA binding protein activity and provide a paradigm-shift for miRNA function as currently understood.

Introduction

Altered differentiation is a common feature of hematologic malignancies, including blast crisis chronic myeloid leukemia (CML). CML is a clonal disorder of the pluripotent

hematopoietic stem cell that is characterized by presence of the t(9;22)(q34;q11) chromosomal translocation. This sole genetic abnormality produces a constitutively active fusion tyrosine kinase known as $p210^{BCR/ABL}$ that is responsible for oncogenic transformation. CML disease is clinically characterized by two distinct phases. Chronic phase (CML-CP) is a prolonged myeloproliferative disorder in which myeloid precursors demonstrate enhanced survival but no change in the capacity for terminal myeloid differentiation. This initial phase, while variable in duration, can progress into a rapidly fatal blast crisis (CML-BC) phase, characterized by the inability of myeloid progenitors to differentiate into mature neutrophils (Calabretta and Perrotti, 2004). While enhanced BCR/ABL expression/activity contributes to CML blastic transformation (reviewed in (Calabretta and Perrotti, 2004)), the molecular mechanisms behind this transition are largely unknown. Our lab has identified several RNA binding proteins (RBPs) whose expression/activity are altered during CML blastic transformation (e.g. hnRNP E2 (Perrotti et al., 2002; Chang et al., 2007; Eiring et al., 2008), hnRNP A1 (Neviani et al., 2005; Eiring et al., 2008) , hnRNP K (Notari et al., 2006; Eiring et al., 2008), and La/SSB (Trotta et al., 2003; Eiring et al., 2008)). These RBPs bind to mRNA in a sequence-specific manner and post-transcriptionally alter the processing, export, or translation of factors that code for critical cell regulators, resulting in enhanced survival, proliferation, and differentiation blockade in CML-BC progenitors. In particular, the block of differentiation during CML blastic transformation occurs through hnRNP E2-dependent translational inhibition of the myeloid-specific transcription factor C/EBPα (Perrotti et al., 2002; Chang et al., 2007). Specifically, hnRNP E2 does so by binding to a c-rich element located in the 5' untranslated region (UTR) of *CEBPA* mRNA.

Since the altered regulatory function of RBPs has a profound effect on the phenotype of CML-BC progenitors, it seems likely that microRNA-mediated post-transcriptional gene

regulation may also play a role. Similar to RBPs, miRNAs bind to mRNA in a sequence-specific manner. Through complementary binding to their mRNA targets, miRNAs regulate gene expression by inducing mRNA cleavage, increasing mRNA decay, or repressing mRNA translation (Ambros, 2003; Bartel and Bartel, 2003). Importantly, miRNAs regulate critical cell functions such as hematopoietic differentiation (Chen et al., 2004; Chen and Lodish, 2005), and are implicated in the development and progression of several types of cancers (Garzon et al., 2006), including hematologic malignancies (Calin et al., 2007; Garzon et al., 2007; Hagan and Croce, 2007). In particular, miR-223 was recently found to regulate granulopoiesis of normal cells, acute promyelocytic leukemia (APL) cells (Fazi et al., 2005), and acute myeloid leukemia (AML) cells (Fazi et al., 2007). Nevertheless, a role for miRNAs in CML is only recently being elucidated (Scherr et al., 2007; Venturini et al., 2007). This study focuses on the role of miRNAs in CML disease progression and provides evidence of a novel function for these tiny RNA molecules as direct inhibitors of RNA binding protein activity.

Results

To initially address the role of miRNAs in CML, we used preliminary microarray analysis to identify miRNAs significantly upregulated or downregulated in both BCR/ABL-expressing myeloid cell lines compared to untransformed parental cells and in CML-BC^{CD34+} bone marrow (BM) cells compared to CML-CP^{CD34+} myeloid progenitors (data not shown). Among the downregulated miRNAs, we focused on miR-223 because of its reported role in normal and leukemic myelopoiesis (Fazi et al., 2005; Fazi et al., 2007; Garzon et al., 2007), miR-15a/16-1 because of their reported role as tumor suppressors (Calin et al., 2005; Cimmino et al., 2005), and miR-328, because although it was highly deregulated, there was a lack of literature regarding its targets and function.

Validation of microarray data by Northern blot and real-time PCR analyses. Consistent with the results of our microarray data, Northern blot analysis demonstrated decreased levels of miR-223, miR-15a, miR-16-1, and miR-328 in BCR/ABL-expressing 32Dcl3 myeloid precursor cells (32D-BCR/ABL) compared to untransformed parental controls (Figure 1A). Treatment of 32D-BCR/ABL cells with the tyrosine kinase inhibitor imatinib rescued expression of these miRNAs, but not in the imatinib-resistant 32D-BCR/ABL(T315I) cell line, indicating that downregulation of these miRNAs is dependent upon BCR/ABL tyrosine kinase activity. Similar results were obtained in the CML-BC-derived K562 cell line (Figure 1B) and in lineage-negative (Lin^-) mouse BM cells retrovirally transduced with the $p210^{BCR/ABL}$ oncoprotein (Figure 1C).

We next analyzed miRNA expression in $CD34^+$ BM cells isolated from myeloid CML-BC (n=7) patients compared to CML-CP (n=4) patients by real-time PCR analysis (Figure 1D). Consistent with results in cell lines, miR-16-1 and miR-328 levels were significantly reduced in $CML\text{-}BC^{CD34+}$ compared to $CML\text{-}CP^{CD34+}$ samples, and while no significant change was found for miR-15a, we unexpectedly detected a significant increase in miR-223 expression in $CML\text{-}BC^{CD34+}$ compared to $CML\text{-}CP^{CD34+}$ samples (discussed below).

hnRNP E2 physically associates with miR-223 and miR-328 in vitro. In the process of identifying putative mRNA targets for miR-328, we realized that both the mature and precursor forms of this miRNA share sequence and structural similarity with the hnRNP E2-binding element found in the 5' UTR of *CEBPA* mRNA (Figure 2A; (Perrotti et al., 2002)). Interestingly, miR-223 also shares sequence similarity with the *CEBPA* uORF, although to a lesser extent (Figure 2A). To initially address whether these miRNAs could bind to hnRNP E2, we performed *in vitro* binding reactions with both purified recombinant MBP-tagged hnRNP E2 (Chang et al., 2007) and endogenous hnRNP E2 from BCR/ABL-expressing myeloid precursor cell lines with synthetic

RNA oligonucleotides (rODNs) corresponding to: 1) the mature miR-223 and miR-328 sequences, 2) the 5' UTR of *CEBPA* mRNA containing the hnRNP E2 binding element (*CEBPA* uORF; positive control), and 3) a mature miR-330 sequence that shares no homology with the *CEBPA* uORF (negative control). As depicted in Figures 2B and 2C, only the *CEBPA* uORF positive control and the miR-223 and miR-328 RNA sequences could efficiently bind to recombinant and endogenous hnRNP E2, whereas the miR-330 negative control and other C-rich miRNAs (data not shown) were unable to form these interactions. Validating the specificity of this interaction, cold excess miR-328 competed for binding of hnRNP E2 to the *CEBPA* uORF and vice versa. UV-crosslinking validated that the MW of the binding partner from protein lysates of BCR/ABL-expressing cells was the correct size for hnRNP E2, and this binding partner was also detected in parental 32Dcl3 cytoplasmic lysates harboring a Flag-tagged hnRNP E2 (32D-Flag-E2) protein (Figure 2D). Based on the affinity of hnRNP E2 for miR-328 and the downregulation of this miRNA in BCR/ABL-expressing cell lines and primary CML-BC patient samples, it is possible that this miRNA interferes with the RNA binding function of hnRNP E2 and that its downregulation is required for transition of disease from CP to BC.

Effects of miR-223, miR-15a/16-1, and miR-328 overexpression on growth and viability of parental and BCR/ABL$^+$ cell lines. To gain further insight into the function of these miRNAs in CML pathogenesis, we prepared retroviral vectors for forced expression of either miR-223, miR-328, or miR-15a/16-1 (which are expressed as a bicistronic miRNA transcript) in parental and BCR/ABL-expressing 32Dcl3 and K562 cells and analyzed the effects of these miRNAs on their growth, viability, and clonogenic potential in methylcellulose medium. None of these miRNAs had a significant affect on growth or viability of parental or BCR/ABL-expressing cells (Figure 3A). While miR-223 expression had no effect on the clonogenic potential of parental or

BCR/ABL$^+$ cells, miR-328 and miR-15a/16-1 expression significantly reduced the clonogenic potential of 32D-BCR/ABL and K562 cells (Figure 3B) with no affect on that of parental 32Dcl3 controls (data not shown).

Effects of miR-223 and miR-328 on G-CSF-induced granulocytic maturation of parental and BCR/ABL-expressing 32Dcl3 cells and primary CML-BC patient samples. Based on the sequence similarity of miR-223 and miR-328 with the *CEBPA* hnRNP E2 binding site, we next analyzed the effect of these miRNAs on G-CSF-induced granulocytic maturation of parental and BCR/ABL-expressing myeloid precursor cells. Consistent with the results of Fazi et al. (Fazi et al., 2005), miR-223 overexpression enhanced the kinetics of differentiation in normal 32Dcl3 myeloid precursor cells, as demonstrated by the presence of terminally-differentiated neutrophils after only three days of culture in the presence of G-CSF, whereas control cells showed signs of terminal differentiation after five days of culture with G-CSF. Interestingly, miR-328 expression had no effect on the differentiation status of normal myeloid progenitors (Figure 4). However, while uninfected and vector-infected 32D-BCR/ABL cells showed no signs of granulocytic differentiation, both miR-223 and miR-328 overexpression rescued differentiation of these cells in response to G-CSF (Figure 5A). In support of this interpretation, C/EBPα protein levels were elevated in both miR-223- and miR-328-overexpressing cells when compared to uninfected and vector-infected controls as detected by Western blot analysis (Figure 5B). These results are consistent with our hypothesis that these miRNAs, particularly miR-328, are capable of inhibiting the function of hnRNP E2 by directly binding to and interfering with hnRNP E2-*CEBPA* mRNA interactions. miR-328 induced higher levels of C/EBPα protein compared to miR-223 in three independent experiments, consistent with the presence of multiple hnRNP E2 binding sites in the precursor and mature forms of miR-328 (Figure 1A).

To determine if similar results could be achieved in primary CML-BC^{CD34+} patient samples, we again used retroviral infection to overexpress either miR-223 or miR-328 in CML-BC^{CD34+} bone marrow cells and analyzed their ability to differentiate in response to human recombinant G-CSF *in vitro*. As expected based on our results in cell lines and its downregulation in CML-BC patient progenitors, miR-328 overexpression resulted in terminal neutrophilic maturation of primary CML-BC patient samples in response to G-CSF (Fig. 6A); control uninfected cells retained the blast cell phenotype. However, while miR-223-overexpressing blast crisis cells showed initial signs of maturation at days five and seven of G-CSF culture, the final cell population resembled that of a more mononuclear phenotype (Figure 6) with apparent inhibition of granulocytic differentiation, consistent with the upregulation of miR-223 that we observed in primary CML-BC versus CML-CP patient samples (Figure 2E). In support of this interpretation, a recent publication using a miR-223 knockout mouse model demonstrated that loss of miR-223 resulted in neutrophilia and enhanced the killing activity of mature neutrophils *in vivo* (Johnnidis et al., 2008), suggesting an inhibitory role for miR-223 in this pathway. Interestingly, C/EBPα protein levels were elevated in both miR-223- and miR-328- overexpressing CML-BC cells compared to controls (Figure 6B), suggesting that the inhibitory effect of miR-223 on granulcytic maturation of blast crisis progenitors is downstream of C/EBPα, and is consistent with our hypothesis that these miRNAs inhibit hnRNP E2 activity.

Discussion

In the current study, we identify BCR/ABL-mediated downregulation of miR-16-1 and miR-328 in both BCR/ABL-expressing cell lines compared to untransformed controls and in CML-BC compared to CML-CP primary patient bone marrow (BM) samples. While miR-15a and miR-223 were found significantly downregulated in BCR/ABL-expressing cell lines, there

was no significant difference in miR-15a expression in CML-BC versus CML-CP samples, and miR-223 expression was found significantly upregulated during CML blastic transformation. We are currently in the process of reconciling the differences in miR-15a and miR-223 expression in cell lines versus primary patient samples. However, we hypothesize that since miR-16-1 is downregulated while miR-15a is left unchanged in CML-BC primary patient samples, there may be a defect in precursor miRNA processing. miR-15a and miR-16-1 are transcribed as a single primary transcript from the same gene, and perhaps an unknown factor (for instance an RBP) may be responsible for the proper processing of miR-16-1 but not miR-15a. This kind of direct interaction between miRNAs and RBPs is not unprecedented. hnRNP A1, another RBP that, like hnRNP E2, binds to mRNA in a sequence-specific manner and is upregulated during CML blastic transformation, was found to directly bind precursor miR-18a (pre-miR-18a) in order to regulate its biogenesis (Guil and Caceres, 2007). miR-18a is expressed as part of the miR-17-92 cluster, an oncogenic miRNA cluster upregulated in several types of cancers (Dews et al., 2006; He et al., 2005), and was recently reported to be upregulated by BCR/ABL (Venturini et al., 2007). While miR-223 was significantly downregulated in 32D-BCR/ABL cells compared to parental controls, it was significantly upregulated in CML-BC versus CML-CP patient BM samples. Accordingly, we found that ectopic miR-223 enhanced G-CSF-induced maturation of 32D-BCR/ABL cells (consistent with the results of Fazi et al.2005), but inhibited that of CML-BC^{CD34+} patient samples (consistent with the results of Johnidis et al. 2008). While additional tests will be required, we hypothesize that these incongruent results are due to the *unipotent* nature of the 32Dcl3 and NB4 cell lines used by our laboratory and Fazi et al., respectively, and the *pluripotent* nature of the CML-BC^{CD34+} and lineage-negative miR-223 knockout mouse cells used by our laboratory and Johnidis et al., respectively. Unipotent 32Dcl3 and NB4 cells are only

capable of differentiating into neutrophils, whereas pluripotent $CD34^+$ BM cells and lineage-negative mouse BM cells are capable of differentiating into all blood cell lineages. It is possible that the function of miR-223 differs in these two environments. The fact that miR-223 was upregulated in CML-BC^{CD34+} cells compared to CML-CP^{CD34+} cells and inhibited their differentiation in response to G-CSF, and yet was still capable of increasing C/EBPα protein levels in CML-BC cells, suggests that the inhibitory role of this miRNA on granulocytic differentiation of pluripotent hematopoietic stem cells is downstream of C/EBPα in the granulcytic differentiation pathway.

Importantly, we provide evidence that hnRNP E2 can bind directly and specifically to miR-223 and, to a greater extent, miR-328 *in vitro*. Furthermore, in BCR/ABL^+ cell lines and primary CML-BC^{CD34+} patient samples, we show that forced expression of miR-328 results in enhanced neutrophilic maturation of differentiation-arrested BCR/ABL^+ cell lines and primary CML patient samples in response to G-CSF. In addition, our data show that both miR-223 and, to a greater extent miR-328, are capable of interfering with the translation-inhibitory activity of hnRNP E2, as indicated by increased levels of C/EBPα protein upon ectopic expression of either miRNA. These data demonstrate for the first time that miRNAs are capable of directly interfering with the RNA binding activity of RBPs and represents a paradigm shift for miRNA function as currently understood today (see model in Figure 7). Interactions of this type may represent novel therapeutic targets for diseases involving aberrant miRNA or RBP expression, including the treatment of blast crisis chronic myelogenous leukemia.

Materials & Methods

Materials and methods for this study can be found in Chang et al., 2007 for cell lines, clonogenic assays, and differentiation assays, and Chen et al., 2004 for preparation of miRNA vectors.

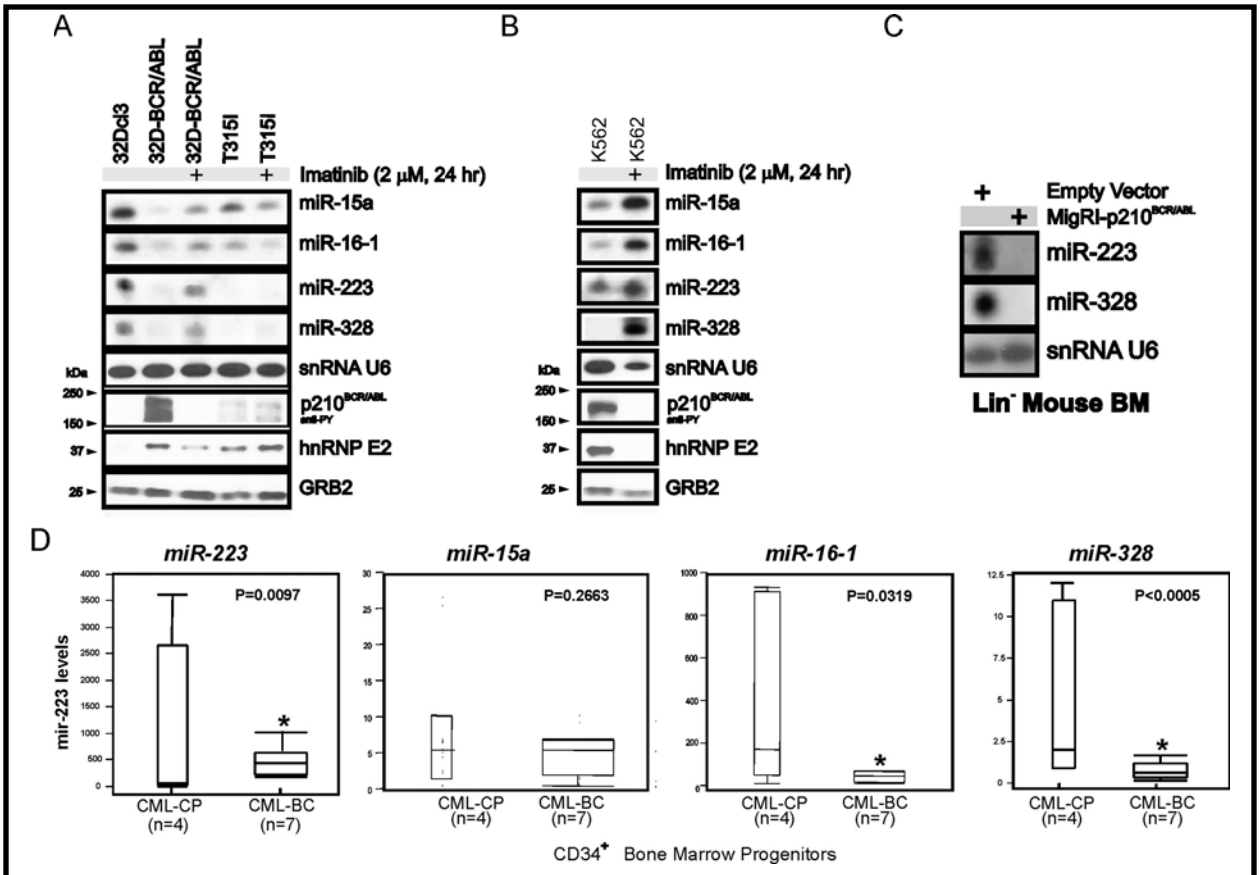

Figure 1. ***Expression of miR-223, miR-15a, miR-16-1, and miR-328 in BCR/ABL$^+$ cell lines and patient-derived CML bone marrow cells.*** Northern blot analysis shows expression of miR-223, miR-15a., miR-16-1, and miR-328, and Western blot analysis shows expression of $p210^{BCR/ABL}$ and hnRNP E2 in (**A**) parental and untreated or imatinib-treated BCR/ABL-expressing 32Dcl3 cells and untreated or imatinib-treated (T315I)BCR/ABL-expressing 32Dcl3 cells that are resistant to imatinib therapy, (**B**) untreated and imatinib-treated K562 cells, and (**C**) vector- and $p210^{BCR/ABL}$-transduced lineage-negative mouse bone marrow cells. (**D**) Real-time PCR shows expression of miR-223, miR-15a, miR-16-1, and miR-328 in $CD34^+$ bone marrow specimens from patients in CML-CP (n=4) compared to CML-BC (n=7). snRNA U6 levels were used for normalization of Northern blot and real-time PCR experiments and GRB2 levels were used for normalization of Western blots.

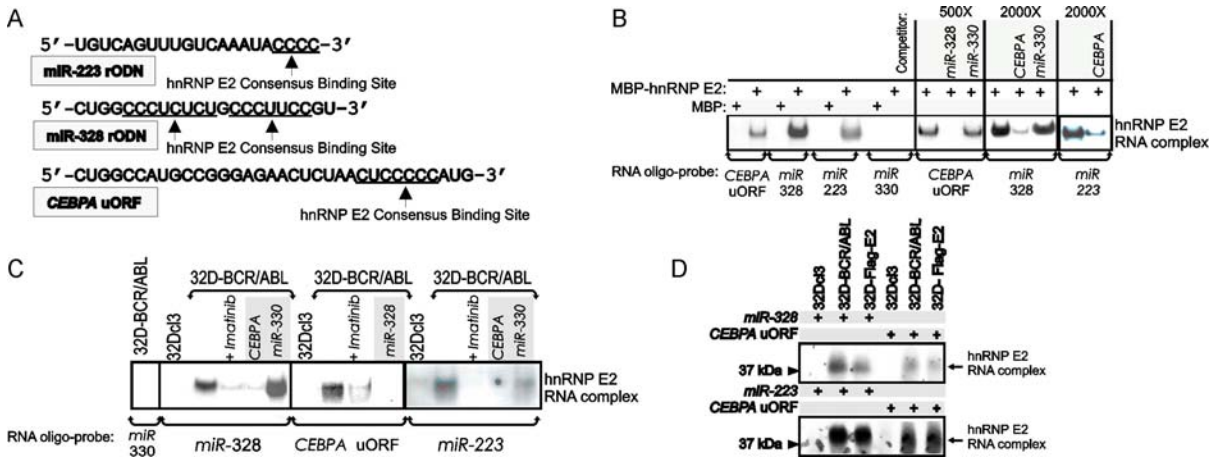

Figure 2. ***Interaction of miR-223 and miR-328 with recombinant and endogenous hnRNP E2.***
(**A**) Representation of the oligoribonucleotides containing the hnRNP E2 consensus binding site located on miR-223 (miR-223 rODN), miR-328 (miR-328 rODN), and the *CEBPA* uORF spacer region (*CEBPA* uORF). (**B**) REMSA shows binding of P^{32}-labeled oligoribonucleotides (rODN) representing miR-223, miR-328, the *CEBPA* uORF (positive control), and a miR-330 sequence that shares no homology with *CEBPA* (negative control), to recombinant MBP-tagged hnRNP E2 compared to MBP alone. 500-2000X cold unlabeled competitor RNA was used to validate the specificity of the interaction. (**C**) REMSA shows binding of the same P^{32}-labeled rODNs to a protein of single molecular weight from cytoplasmic protein lysates isolated from parental and untreated or imatinib-treated BCR/ABL-expressing 32Dcl3 cell lines. The specificity of this interaction was again validated using cold competitor RNA. (**D**) UV-crosslinking of binding partners using cytoplasmic protein lysates of 32Dcl3, 32D-BCR/ABL, and 32D-Flag E2 cell lines with miR-223, miR-328, and *CEBPA* uORF were fractionated on native SDS-PAGE and analyzed by autoradiography.

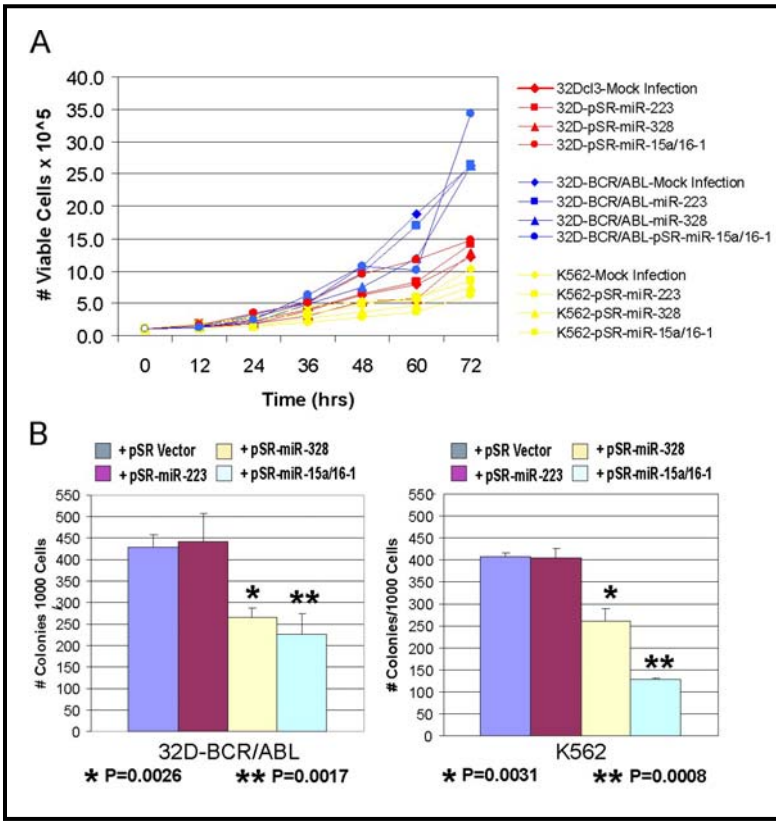

Figure 3. ***Effect of miR-223, miR-15a, miR-16-1, and miR-328 overexpression on growth, viability, and clonogenic potential of normal and BCR/ABL-expressing cells.*** (**A**) Retroviral-mediated expression of miR-223, miR-15a, miR-16-1, or miR-328 had no effect on the growth or viability of parental or BCR/ABL^+ cell lines. (**B**) While miR-223 had no effect on clonogenic potential of BCR/ABL^+ cells, miR-15a/16-1 and miR-328 significantly inhibited the clonogenic potential of both 32D-BCR/ABL and K562 cell lines.

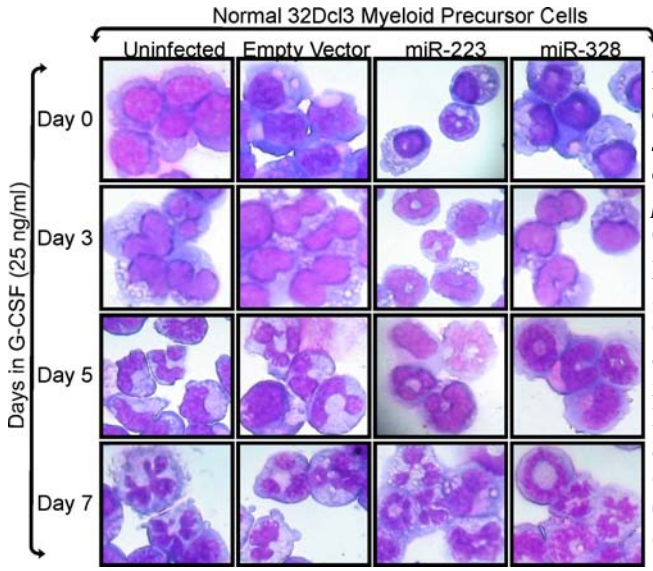

Figure 4. ***Effect of retroviral expression of miR-223 and miR-328 on granulocytic differentiation of normal 32Dcl3 myeloid precursor cells in response to G-CSF.*** Parental, empty vector pSUPER.retro.neo.GFP, pSR-miR-223-expressing, and pSR-miR-328-expressing 32Dcl3 cells were analyzed for neutrophilic maturation in response to G-CSF *in vitro*. Morphologic differentiation was assayed by Wright-Grunwald/Giemsa staining on days 0, 3, 5, and 7 after addition of G-CSF to the medium.

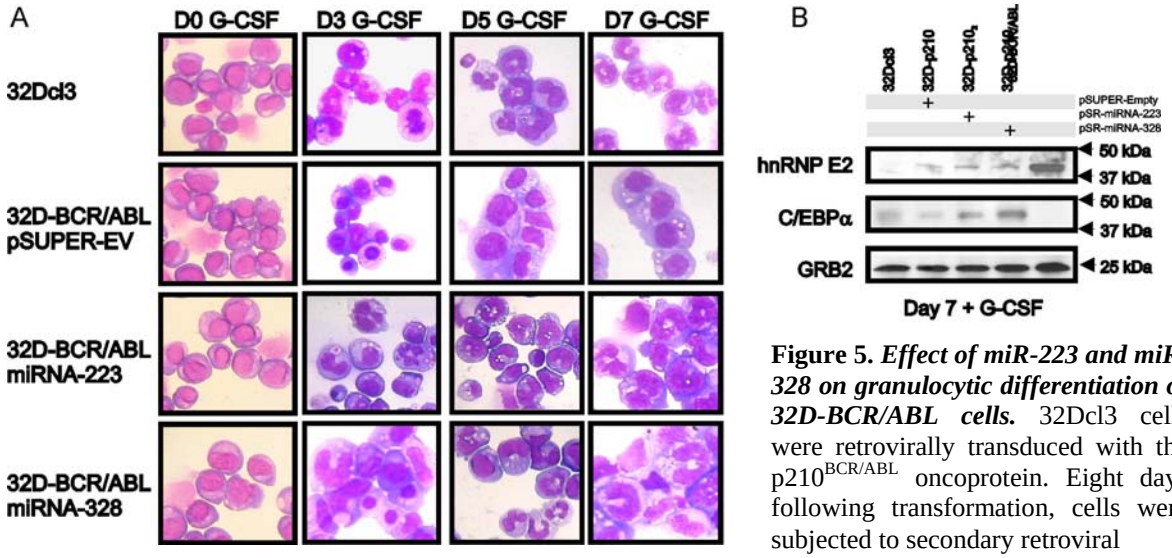

Figure 5. ***Effect of miR-223 and miR-328 on granulocytic differentiation of 32D-BCR/ABL cells.*** 32Dcl3 cells were retrovirally transduced with the $p210^{BCR/ABL}$ oncoprotein. Eight days following transformation, cells were subjected to secondary retroviral infection with either pSUPER empty vector, pSR-miR-223, or pSR-miR-328, and subject to culture in the presence of G-CSF. (**A**) Morphologic differentiation was assayed by Wright /Giemsa staining on days 0, 3, 5, and 7 following G-CSF administration. (**B**) Western blots demonstrate levels of hnRNP E2 and C/EBPα protein expression on the indicated days. GRB2 levels were analyzed for normalization.

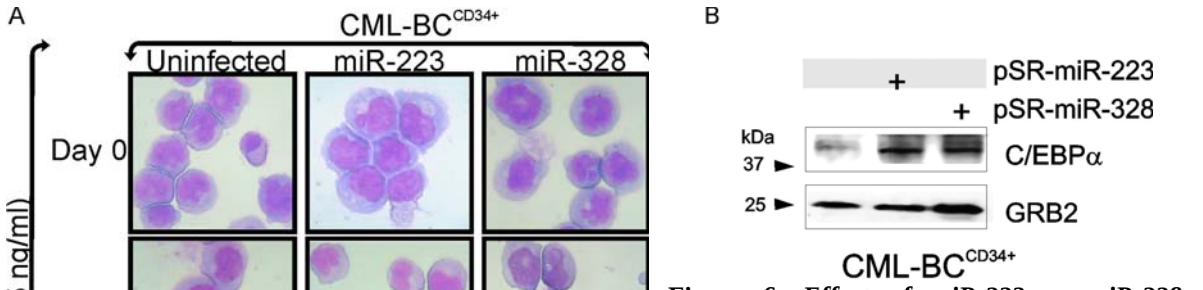

Figure 6. ***Effect of miR-223 or miR-328 expression on granulopoeisis of CML-BC^{CD34+} bone marrow progenitors.*** Primary myeloid CML blast crisis patient bone marrow cells were cultured and magnetically sorted for the CD34^{+}. Cells were subsequently used for retroviral transduction with miR-223, miR-328, or uninfected and analyzed for differentiation in response to human recombinant G-CSF *in vitro.* (**A**) Morphologic differentiation was assayed by Wright/Giemsa staining on days 0, 5, 7, and 10 following G-CSF administration. (**B**) Western blot analysis demonstrates C/EBPα protein levels for each group at day 10 following G-CSF administration.

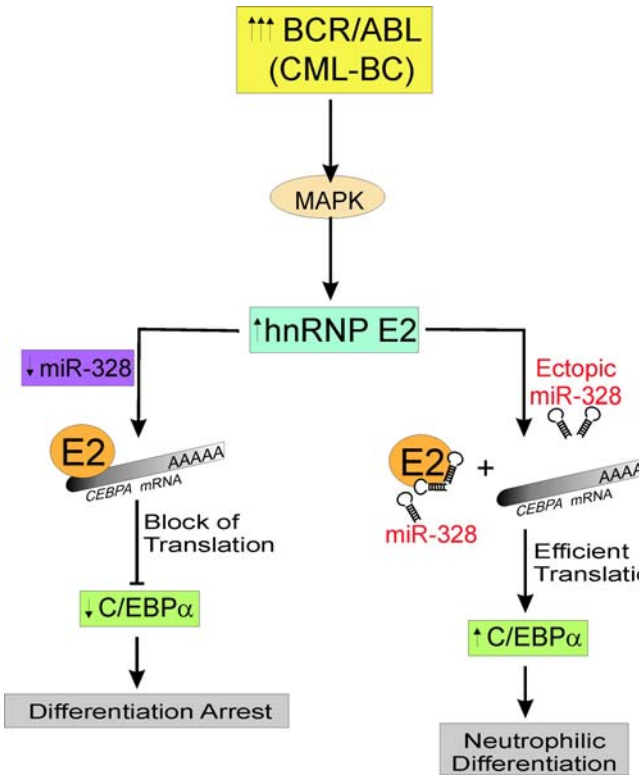

Figure 7. ***Model of the role played by miR-328 in granulocytic maturation of BCR/ABL^{+} myeloid progenitors during CML blast crisis.*** Our lab previously demonstrated that BCR/ABL tyrosine kinase activity blocks myeloid differentiation through the MAPK-dependent phosphorylation and activation of hnRNP E2 translation-regulatory activity, resulting in translational blockade and loss of C/EBPα protein expression(Perrotti et al., 2002; Chang et al., 2007). Our model suggests that during differentiation arrest as observed in CML-BC, BCR/ABL tyrosine kinase activity downregulates miR-328 expression (left side), thus allowing the negative-regulatory activity of hnRNP E2 on *CEBPA* mRNA and blocking differentiation of CML-BC progenitors. Forced expression of miR-328 (right side), however, is capable of inhibiting the RNA binding activity of hnRNP E2. Similar to the effects of imatinib and CI-1040 on repression of hnRNP E2 activity and restoration of granulocytic differentiation (Chang et al., 2007), miRNA-mediated inhibition of hnRNP E2 function releases *CEBPA* mRNA from the translational block of hnRNP E2, thus allowing the following: 1) expression of the myeloid transcription factor C/EBPα, 2) upregulation of the G-CSF receptor, and 3) concomitant neutrophilic differentiation in response to G-CSF. These data point to a new function for miRNAs act as decoy molecules that can restore granulocytic maturation of differentiation-arrested BCR/ABL^{+} myeloid progenitors by directly interfering with the function of hnRNP E2.

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