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miR-125a regulates cell cycle, proliferation, and apoptosis by targeting the ErbB Pathway in Acute Myeloid Leukemia

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Abstract

microRNA profiling of Acute Myeloid Leukemia patient samples identified miR-125a as being decreased. Current literature has investigated miR-125a's role in normal hematopoiesis but not within Acute Myeloid Leukemia. Analysis of the upstream region of miR-125a identified several CpG islands. Both precursor and mature miR-125a increased in response to a de-methylating agent, Decitabine. Profiling revealed the ErbB pathway as significantly decreased with ectopic miR-125a. Either ectopic expression of miR-125a or inhibition of ErbB via Mubritinib resulted in inhibition of cell cycle proliferation and progression with enhanced apoptosis revealing ErbB inhibitors as potential novel therapeutic agents for treating miR-125a-low AML.

Keywords

microRNA-125a; methylation; cell cycle; apoptosis; Mubritinib

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disease marked by a highly variable clinical course and response to therapy. Developing new AML treatments is crucial for increasing survival rate and decreasing cytotoxicity. microRNA's (miR) have entered the spotlight for being involved in the pathogenesis of numerous diseases. microRNAs are non-protein coding RNAs 19–24 nucleotides in length and have recently been highlighted to play a role in normal and malignant hematopoiesis. From miRNA expression studies, it has been found that miRs can regulate maintenance and differentiation of cells in hematopoiesis.(1)

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For example, *miR-130*, *miR-196a* and *miR-196b* are expressed in hematopoietic stem cells and decrease when differentiating towards lymphoid lineages (2) while *miR-25*, *miR-221*, and *miR-223* are increased when a stem cell is directed towards myeloid cells (2). Garzon et al have demonstrated irregular expression of miRs in AML (3). Another study identified *miR-let-7b* and *miR-let7c*, which regulate RAS, to be decreased in AML resulting from t(8;21) chromosomal translocation (4). Though these miR's are being illustrated to play vital roles in normal and malignant hematopoiesis, the functional roles of these miR's need to be elucidated (1).

In a study where miR expression was analyzed in bone marrow (BM) of newly diagnosed cytogenetically normal AML patients compared to BM of control patients, miR-125a was found to be significantly down regulated miR (3). Though miR-125a was not the highlight of Ramsingh et al. article, from the small RNA sequencing within the study miR-125a expression was decreased in cytogenetically normal AML (5). Currently, it is known that *miR-125a* is expressed highest in hematopoietic stem and progenitor cells (HSPC) and progressively decreases upon differentiation (6). Guo et al demonstrated ectopic expression of *miR-125a* in mouse HSPC demonstrated an increase in reconstitution of blood lineages in bone marrow transplantations lasting up to five months as well as HSPC self-renewal (6). Interestingly, ectopic expression of *miR-125a* within common lymphoid progenitors, common myeloid progenitors, granulocyte and macrophage progenitors, and MEP did not result in self-renewal or maintenance of blood lineages. Further analysis illustrated an increase of HSPC to differentiate into myeloid cells and a decrease in lymphoid cells. (6) Within a second study by Gerrits et al, over-expression of miR-125a in mouse HSPC increased competitive advantage and promoted myeloid differentiation, similar to Guo et al. However, unlike Guo et al, miR-125a overexpression did not result in primitive bone marrow maintenance in secondary bone marrow transplants. (7) From these two main studies on the role of miR-125a it is seen that the role of miR-125a in normal hematopoiesis remains inconclusive and furthermore undefined in AML.

Within cancer, miR-125a is decreased in breast cancer, gastric cancer, and medulloblastoma promoting the progression of the disease (8–10). Previous findings within breast and gastric cancer demonstrate that upon re-expression of miR-125a results in decreased cell proliferation, migration, and cell death therefore suggesting that re-expression of miR-125a could be a potential therapeutic (9, 10). Knowing that significantly decreased miR-125a promotes breast cancer, gastric cancer, and medulloblastoma supports the possibility of decreased miR-125a aids in the progression of AML. Therefore within these studies, the functional role of miR-125a was dissected to elucidate if miR-125a could aid in the pathogenesis of AML and if it could be utilized as a new therapeutic for AML.

Materials and Methods

Statistical Analysis

GraphPad Prism 6 was utilized to graph and determine significance. T-Test (parametric test, unpaired test) was used to determine significance.

National Cancer Institute the Cancer Genome Atlas Data Portal Analysis

Data files were downloaded from: <https://tcga-data.nci.nih.gov/tcga/>. Files were downloaded from Data Matrix for LAML level 3 for miRNASeq and Clinical Data. Script was written to combine miR-125a reads per million to its corresponding clinical information into one excel file. For low and high miR-125a expression, miR-125a expression was sorted from low to high miR-125a expression and then the median was calculated. Values beneath median were considered low miR-125a expression whereas values above median were considered high miR-125a expression.

Cell culture

NB4 cells were cultured in RPMI-1640 with L-Glutamine with 10% fetal bovine serum and 1X PSF. MV4-11 and K562 were cultured in Iscove's Modified Dubecco's Medium (IMDM) with Hepes and L-Glutamine with 10% fetal bovine serum and 1X PSF. HL60 cells were IMDM with Hepes and L-Glutamine with 20% fetal bovine serum and 1X PSF.

Lentiviral transduction

NB4 cells were transduced with miRIDIAN shMIMIC has-miR-125a lentiviral particles (Thermo Scientific #C-300624-05-0005). miR-125a-5p lentivirus particles at a multiplicity of infection (MOI) between 10 and 15 were added to the NB4 cells at 37°C. For controls, lentivirus particles expressing GFP were used at similar MOIs. The cells were infected for 15 hours and then recovered in culture medium. Following puromycin selection (final concentration 2µg) high transduction and expression of *miR-125a-5p* was confirmed by analysis of mature miR-125a expression using RT-qPCR.

Inhibitors

NB4 cells were pre-treated with Decitabine (Selleck Chemicals #S1200) or DMSO for control 24 hours before miR isolation or DNA isolation. NB4 cells or HL60 cells were pre-treated with Mubritinib (Selleck Chemicals #S2216) or DMSO for control 24 hours before miR isolation, MTT assay, and BrdU. NB4 cells were pre-treated with Mubritinib for 96 hours prior to Cd11b and Annexin-V analysis.

miRNA isolation and RT-qPCR

miRNA was isolated from cell lines using miRNeasy Mini Kit (Qiagen) following the manufacturer's instructions. For analysis of mature miR-125a, cDNA was made utilizing RT² miRNA First Strand Kit (SA Bioscience # 331401). cDNA was made from CD34+ (Stem Cell Technologies #RNA-BM003C) and CD33+(Stem Cell Technologies #RNA-BM006C) RNA obtained from Stem Cell Technologies using the RT² miRNA First Strand Kit (SA Bioscience # 331401). Primers used in mature miR-125a analysis were hsa-miR-125a-5p (Qiagen #MPH00022A-200) and RNU-6 (Qiagen# MPH01653A-200). For analysis of precursor miR, cDNA was made utilizing the miSCRIPT RT II Kit (Qiagen # 218160). Primers used for analysis of precursor miR-125a were Hs_mir_125_1_PR (Qiagen# MP00000455), Hs_RNU6_2_1 (Qiagen miSCRIPT PCR Starter Kit # 218193), and 10X miSCRIPT Universal Primer (Qiagen miSCRIPT PCR Starter Kit# 218193).

Results were normalized to the RNU-6 (sets to 1) values using the $2^{-(\Delta\Delta C(T))}$ method (11).

Protein isolation and Western blots

Protein was isolated from NB4 cells following pre-treatment with Mubritinib using RIPA buffer (0.05M Tris, 0.15M NaCl, 0.85% Triton X-100, 0.05% SDS, phosphatase inhibitor, and protease inhibitor). For Western blots, the following antibodies from Cell Signaling were used: HER2/ErbB2 (2165), P-HER2/ErbB2 (Y1221/1222) (#2243), AKT1 (#2938), P-AKT1 (S473) (#4058), p44/42 MAPK (#4695), P-p44/42 MAPK (T202/Y204) (#4370), and β -tubulin (#2128S). Blots were visualized using Electrochemiluminescence.

Bisulfite Sequencing and global methylation

NB4 cells were pre-treated with 10 μ M of Decitabine for 24 hours prior to bisulfite sequencing analysis. For in depth details for bisulfite sequencing please see supplemental methods. NB4 cells were pre-treated with 10 μ M of Decitabine for 24 and 48 hours before global methylation analysis by Epigenetek Methylflash Methylated DNA quantification kit (Cat#P-1034-48) following protocol provided.

MTT Assay

Transduced NB4 cells or NB4 cells pre-treated for 24 hours with Mubritinib were analyzed for cell proliferation following the instruction manual provided within the Vybrant MTT Cell Proliferation Assay Kit (Invitrogen #V13154).

Flow Cytometry

Flow cytometry analyses were performed using FACS Calibur (BD Biosciences). Cell cycle progression of cells was conducted following the manufacturer's instructions included within the FITC BrdU Flow Kit (BD Pharmingen #51-2354AK). For cell death analysis, cells were stained with APC Annexin-V (BD Pharmingen #550474) and Propidium Iodide (Invitrogen #P3566) following the manufacturer's instructions. For CD11b analysis, cells were stained with PE CD11b (BD Bioscience #561689) and analyzed via flow cytometry.

Gene expression profiling using microarrays

For in depth methods on profiling please see supplementary methods.

NB4 human leukemic xenograft model and Histology

3 $\times 10^6$ NB4 cells were subcutaneously injected into the left flank of twenty-four NOD.CB17-Prkdc scid/J mice (Jackson Laboratory #001303). Mice were randomized for treatment with Mubritinib or vehicle prior to start of experiment. Seven mice from the Mubritinib group and ten mice from the vehicle group formed tumors and progressed to the treatment stage. Two treatments of 20mg/kg Mubritinib or vehicle (DMSO) was given on the first day as a loading dose followed by daily treatment of 20mg/kg of Mubritinib or vehicle via oral gavage. Tumor measurements were conducted throughout the course of study and tumor volume was calculated by the following equation:

tumor volume = $(A \times B^2) \times \frac{1}{2}$ where A equals the longer dimension and B equals the shorter dimension. The final two tumor measurements and the tumor collection were performed in a blinded manner. After tumor excision, tumors were paraffin embedded, sectioned and stained for H&E, Ki-67, and caspase-3 in accordance with standard immunohistochemistry protocols by the MMCRI histology core.

RESULTS

Dissecting miR-125a expression in clinical samples

Previously, Garzon et al showed miR-125a expression was decreased in cytogenetically AML (3). To further understand miR-125a expression in AML, data from the National Cancer Institute the Cancer Genome Atlas (TCGA) Data Portal was utilized. By using this site, expression levels of miR-125a were analyzed within 167 AML patients in a range of clinical aspects (Supplemental Figure 1A). From miR-125a expression analysis, the majority of the patient samples had between 1 and 250 reads per million as visible in the histogram (Supplemental Figure 1B). No difference between miR-125a expression and gender or age of diagnosis was found (Supplemental Figure 1C and D). Though a difference appears between ethnicity, there was only one patient within the Hispanic or Latino group (Supplemental Figure 1E). Interestingly, there were several clinical features altered significantly with low miR-125a expression. Bone marrow blast count decreased significantly whereas leukocytes, promonocytes, monocytes, and platelets significantly increased (Supplemental Table 1). Through analysis of miR-125a expression in relation to French-American-British (FAB) classification, subtype M5 has the lowest miR-125a expression (Supplemental Figure 2). To further dissect low miR-125a classification in AML, cytogenetic and molecular abnormalities were analyzed, however due to the complexity of the disease, no specific classification could be identified (Supplemental Tables 2 and 3). Many of the patients had their own unique molecular and cytogenetic abnormality (Supplemental Tables 2 and 3). miR-125a expression was most decreased in favorable and intermediate prognostic and associated with decreased survival (Supplemental Figure 3A and B). Although the TCGA is useful for analysis of miR-125a expression and correlation to clinical samples, at this time a healthy control was not available.

Restored miR-125a expression causes decreased cell proliferation, cell cycle progression, and enhanced apoptosis

Several leukemic cell lines were screened in order to determine miR-125a expression status. MV4-11 (Biphenotypic B Myelomonocytic Leukemia), K562 (Chronic Myeloid Leukemia), and HL60 (Acute Promyelocytic Leukemia) had decreased miR-125a expression in comparison to CD34+ or CD33+ cells (Figure 1A). miR-125a expression was most significantly decreased in human acute promyelocytic leukemia (APL) NB4 cells containing t(15;17) in comparison to either CD34+ or CD33+ cells (Figure 1A). Using the TCGA data, miR-125a expression was analyzed in samples containing PML-RAR alpha mutation. Though no difference was seen in patients with PML-RAR alpha mutation and those without this mutation, an in depth look at samples with PML-RAR alpha demonstrate complex heterogeneity (Supplemental Figure 5A and B). Therefore, no conclusion can be made on

the effects of PML-RAR alpha mutation and miR-125a expression. Due to the significant decrease of miR-125a in NB4 cells, these cells were used to identify the effects of restoring miR-125a expression within AML.

To elucidate the role of miR-125a's functional role in NB4 cells, miR-125a was over expressed in NB4 cells (Supplemental Figure 4A and Figure 1B). Confirmation that cells were properly transduced is seen through both GFP positive expression (Supplemental Figure 4A) and miR-125a fold change (Figure 1B). Analysis of cell proliferation via an MTT assay revealed a decrease in cell proliferation in response to overexpression of miR-125a (Figure 1C). Cells started to accumulate at the G0/G1 stage causing a decrease in both the S-phase and G2/M phase when miR-125a was overexpressed (Figure 1D and Supplemental Figure 4B). Cell death analysis via co-staining of PI and Annexin-V on flow cytometry resulted in increased apoptosis as visualized through Annexin-V positive cells (Figure 1E and Supplemental Figure 4C) when miR-125a is overexpressed. Although, K562 and MV4-11 cells did not ectopically express miR-125a at the fold change as NB4 cells (Supplemental Figure 6A), restoring miR-125a expression in additional lines that do not have significantly decreased miR-125a expression results in increased cell proliferation rather than decreased cell proliferation (Supplemental Figure 6B). This outcome may be due to the variation in cytogenetic or molecular abnormalities for these cells lines.

Although pharmacological mimic miR is being developed for treatments, utilization of pharmacological mimic miR as therapy is still evolving. Therefore gaining insight to the molecular basis of miR-125a's silencing is a viable alternative approach to re-express miR-125a in patients who have decreased miR-125a expression.

miR-125a is epigenetically silenced in AML

When considering the molecular mechanisms of miR-125a silencing there are several possible mechanisms leading to decreased suppression of a miR, including microRNA processing (12–14), transcriptional (15), or post-transcriptional (16, 17) regulation. Within the context of AML aberrant methylation has been correlated to decreased gene expression (18, 19). When analyzing the upstream region of miR-125a using PubMed's Gene/Epigenomics Tool, several CpG islands upstream were identified (Figure 2); therefore, miR-125a expression was analyzed in response to a de-methylating agent, decitabine. Mature miR-125a expression increased with increasing amounts of decitabine treatment after 24 hours (Figure 2A) and slightly decreases by 48 hours (data not shown). If miR-125a is transcriptionally expressed, precursor miR-125a should additionally increase in response to decitabine. Correlating to increased mature miR-125a expression, precursor miR-125a expression was further found to increase significantly in response to decitabine treatment at 24 hours (Figure 2B). Global methylation analysis demonstrated that decitabine was resulting in decreased methylation of NB4 cells (Figure 2C). To confirm that increased precursor and mature miR-125a expression was a result of decreased methylation of upstream CpG island methylation bisulfite sequencing was performed. Analysis of a portion of the large CpG Island revealed a decrease of fifty-six percent (Figure 2D). Alterations of the small CpG islands were not visible at the decitabine concentration and time point analyzed (data not shown). Combined results from bisulfite sequencing and miR-125a

expression in response to decitabine treatment indicate that miR-125a is transcriptionally suppressed by methylation. Although miR-125a expression is restored by decitabine treatment, decitabine is resulted in global de-methylation within NB4 cells and therefore, present work was focused on defining miR-125a's role and potential target when re-expressed in AML. By gaining insight towards this pathway, more targeted treatment for low-miR-125a AML could be developed.

miR-125a significantly regulates AML systemically

RNA expression profiling demonstrated systemic changes in biological processes enlightening us on the impact of miR-125a's role in AML. ANOVA results from the gene-level analysis include 812 genes differentially expressed between miR-125a and GFP control groups ($p < 0.05$; fold change > 1.5) (Supplemental Figure 7B, Supplemental Table 4A and 1B). Of these 812 genes, 449 genes were downregulated (Supplemental Figure 7B, Supplemental Table 4A) and 363 probe sets were upregulated (Supplemental Figure 7B, Supplemental Table 4B). A three-dimensional scatter plot from a principal component analysis clearly identifies distinct molecular phenotypes in miR-125a samples and shows no significant outliers in the study set (Supplemental Figure 8). The first principal component (PC1) correlates with our single biological factor, NB4 cell line.

A hierarchical clustering map generated from these 812 significant differentially expressed genes revealed two distinct expression patterns (Supplemental Figure 7A). miR-125a samples lower half of the map and GFP samples in the upper half of the map (genes with increased expression are shown in red and genes with decreased expression in green). The 363 probes/genes in the upper right quadrant of the cluster map are genes that show higher levels of expression in miR-125a samples relative to GFP control samples. The 449 probes/genes left upper quadrant are genes that exhibit lower expression levels in miR-125a samples relative to GFP control samples. Results from the exon-level ANOVA include 2830 alternative spliced genes and 3980 genes that are differentially expressed ($p < 0.05$). Of these, 694 are alternatively spliced and differentially expressed (Figure 7C, Supplemental Table 5A and 5B). Pathway ANOVA recovered 262 KEGG functional groups that were either significantly differentially expressed and/or disrupted in a few genes as a result of treatment ($p < 0.05$ for both differential expression and disruption) (Supplemental Table 6). GO ANOVA yielded 1685 differentially expressed and 3838 disrupted GO categories ($p < 0.05$) (Supplemental Figure 7D–E).

Targeting ErbB pathway reveals potential new therapeutic for AML

When conducting ANOVA pathway analysis, the ErbB pathway was significantly decreased ($p = 0.004$, Supplemental Figure 9 and 10; Supplemental Table 7). Previously, overexpression of miR-125a in human medulloblastoma led to cell growth arrest and apoptosis(8). Further, similar ectopic expressions in gastric cancer cells led to inhibition of proliferation (9). Likewise, overexpression of miR-125a in breast cancer cells led to decreased anchorage dependent growth (10). Within the context of the gastric cancer and breast cancer studies, it was identified that miR-125a regulates these cellular functions through ErbB2, which were validated through 3'UTR luciferase assays. In visualizing the ErbB pathway within KEGG pathway analysis, there is visible decrease in several ErbB

receptors (ErbB-1 and ErbB-3) and many of the downstream effectors (Supplemental Figure 9 and 10; Supplemental Table 7). Although ErbB2 did not show a significant decreased within profiling results, enhanced ErbB pathway has been correlated in related to decreased miR-125a and plays roles in proliferation and survival and is commonly altered within cancer (9, 10, 20). Mubritinib (TAK-165), previously shown to be a selective inhibitor of ErbB2 phosphorylation was tested (21). An apparent decrease in cell proliferation of NB4 cells was observed upon Mubritinib treatments (Figure 3A). Strikingly, Mubritinib treatment of NB4 cells resulted in almost complete inhibition of G1- S-phase transition (Figure 3B). HL60 cells, which have more miR-125a expression, remained unaffected at 24 hours post treatment (Figure 3B). There was a significant increase in cell death as visible by co-staining of Annexin-V and Propidium Iodide (PI) with Mubritinib treatment (Figure 3D). CD11b expression was increased significantly on NB4 cells treated with Mubritinib 96 hour's post-Mubritinib treatment representing enhanced differentiation of NB4 cells (Figure 3E).

In order to correlate the effect of Mubritinib to miR-125a expression levels, additional leukemic lines with a range of miR-125a expression were analyzed. These results demonstrated that K562, which additionally has decreased miR-125a expression, had similar effects on inhibited cell cycle progression (Supplemental Figure 11 C, D, G, and H). MV4-11 and HL60, which do not have decreased miR-125a expression, did not have altered cell cycle progression (Supplemental Figure 11 A, B, E, and F); therefore suggesting that miR-12a expression could indicate the effectiveness of Mubritinib on AML. Cell proliferation and apoptosis effects through inhibition of ErbB2 phosphorylation by Mubritinib have been previously suggested to be via decreased phosphorylation of its downstream effectors AKT and MAPK (21). To examine if Mubritinib was acting through these pathways within NB4 cells, phospho- and total- protein levels were analyzed. Mubritinib treatment highly decreased Erbb2 phosphorylation in NB4 cells (Figure 3F). Furthermore Mubritinib decreased phospho-AKT and phospho-MAPK whereas total protein levels were unaffected (Figure 3F). These results indicate that cellular effects observed via Mubritinib in NB4 cells are via ErbB2-AKT-MAPK signaling axis.

Mubritinib inhibits in vivo tumor growth of NB4 human leukemic xenografts

Due to the dramatic effects of Mubritinib treatment of NB4 cells observed in vitro, we tested Mubritinib in an in vivo model of NB4 cells xenograft in NOD-SCID mice (Figure 4A). Tumors were allowed to grow to approximately 200 mm³ in size before treated with vehicle or Mubritinib, but upon treatment with Mubritinib a significant effect on tumor growth was visible (Figure 4A and B; Supplemental Figure 12A). Histopathological analysis revealed that the majority of the Mubritinib treated mice had large areas of necrosis, which were confirmed by lack of Ki-67 (proliferation) staining (Figure 4C and Supplemental Figure 12B). Caspase-3 staining for cell death was most prominent surrounding the regions of necrosis (Figure 4C and Supplemental Figure 12B). Mice treated with the vehicle also had regions of necrosis, but typically the necrotic regions were not as large as those treated with Mubritinib. These findings therefore suggest that although the tumors may have appeared to be growing at the end there were also massive areas of necrosis and decreased proliferation (Supplemental Figure 12B). From these findings, inhibition of the ErbB pathway could be a new therapeutic for patients with low miR-125a expression.

DISCUSSION

Through re-expressing miR-125a expression in NB4 cells, identified as having significantly decreased miR-125a via stable lentiviral transduction of mimic miR-125a demonstrated decreased cell proliferation, cell cycle progression and enhanced apoptosis. By understanding the molecular mechanism of miR-125a expression could lead to a new therapy for low miR-125a AML. Analysis of the upstream region of miR-125a, treatment of a de-methylating agent, and bisulfite sequencing revealed that miR-125a is suppressed by methylation in AML. Although Decitabine treatment achieved restoration of miR-125a expression at 24 hours there was a slight decrease at 48 hours, suggesting that due to global decreased methylation sustained miR-125a restoration was not achieved. Global methylation analysis demonstrated a decrease at 24 hours with a more significant decrease at 48 hours illustrating Decitabine was functioning properly. Due to decreased global methylation, aberrant expression of genes could occur and therefore genes and pathways altered by miR-125a restoration could lead to more specific therapeutics.

To elucidate potential targets and pathways alterations due to miR-125a overexpression, profiling was conducted. Among the significantly decreased genes were FLT1, MMP-9, IL-32R α and HIP-1. FLT-1 is increased within myelodysplastic syndrome and associated with poor prognosis (22). Further, MMP-9 has been demonstrated to play an important role within invasion of malignant myeloblasts (23). IL-3R α has several studies linking its overexpression to AML by demonstrating its overexpression results in enhanced blast proliferation, increased cellularity, and poor prognosis (24). To combat this, several studies have also demonstrated that inhibition of IL-3R α may be a new potential therapeutic since inhibition leads to cell death of AML cells (25, 26). Lastly, HIP-1 has been demonstrated within prostate and colon cancer to be overexpressed leading to cell survival, which could be playing similar roles within AML (27). As with the decreased genes, several increased genes were of interest such as cathepsin-G, EPX, and SPARC. Within one study, the authors demonstrated that re-expression of cathepsin-G within AML can lead to differentiation, growth inhibition and apoptosis of AML1-ETO cells (28). Although this gene was studied within a specific subtype of AML, cathepsin-G could be furthered analyzed within low miR-125a expressing cells since it was increased significantly and previously decreased within an AML subtype. EPX is known to be a marker of eosinophil (29), suggesting that its increased expression could reflect differentiation of NB4 cells with increased miR-125a expression. Additionally, SPARC has been demonstrated to play a potential role within AML due to its decreased expression (30) and addition of exogenous SPARC leads to growth inhibition of AML cells (30, 31).

From ANOVA pathway analysis, the ErbB pathway was significantly altered, such as decreased ErbB receptors (ErbB1 and ErbB3) and downstream effectors (PI3K, AKT, and Stat5). Though the ErbB pathway has been highly demonstrated within the context of epithelial cancers such as breast and gastric cancer (9, 10), it is just starting to be studied within hematopoietic malignancies. When visualizing fold change values of the ErbB pathway within KEGG pathway analysis, the ErbB pathway appeared to be decreased implicating the potential use of ErbB inhibitors for miR-125a low AML. For targeting the ErbB pathway, ErbB2 inhibitors have been well developed, therefore we tested Mubritinib

(TAK-165), which selectively inhibits ErbB2 phosphorylation (21). Previously, Mubritinib was found to be a potential new treatment for bladder, kidney and androgen-independent prostate cancer due to significant decrease in tumor growth in in vivo xenograft models (21). A profound effect was seen in cell cycle progression and additionally the inhibitor significantly altered cell proliferation, differentiation, and apoptosis. Most strikingly was the lack of effect of the inhibitor on HL60 cells, which do not have decreased miR-125a expression suggesting that inhibition of the ErbB pathway would be specific for low miR-125a AML.

Several recent studies have additionally demonstrated that inhibition of the ErbB pathway may be a potential new therapeutic for leukemia. Irwin et al recently found that small molecular ErbB inhibitors may be an effective new therapeutic in BCR-ABL acute lymphoblastic leukemia due to increased apoptosis and decreased proliferation with ErbB inhibitors (32). Martin-Subero et al discovered that ErbB2 was amplified within a Myelodysplastic Syndrome (MDS) patient who developed AML(33). Further studies by Nordigården et al demonstrate that canertinib, a pan-ERBB inhibitor is effective in both primary samples and within a murine model for treatment of FMS-like tyrosine kinase 3 (FLT3) in patients with acute myeloid leukaemia (AML) (34).

As a result of these novel findings, several insights have been gained. First, decreased miR-125a within AML may give leukemic blasts an advantage. From pathway analysis we demonstrated how analysis of the systemic effects that miR's can have beyond studying a single target. By utilizing pathway analysis in NB4 cells ectopically expressing miR-125a, we have identified a potential new therapeutic target for miR-125a low AML, which could be tested further due to the availability of ErbB pathway inhibitors. Thus, ErbB inhibitors currently being utilized for treating ErbB overexpressing epithelial cancers could be tested in hematopoietic malignancies, such as miR-125a low AML.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Figure 2.

Figure 3.

Figure 4.