Acute myeloid leukemia (AML) with NPM1 mutation is a disease driving genetic alteration with good prognosis. Although it has been suggested that NPM1 mutation induces chemosensitivity in leukemic cells, the underlying cause for the better survival of NPM1 mutated patients is still not clear. Mutant NPM1 AML has a unique microRNA and their target gene (mRNA) signature compared to wild-type NPM1. Dynamic regulation of miRNA–mRNA has been reported to influence the prognostic outcome. In the present study, in silico expression data of miRNA and mRNA in AML patients was retrieved from genome data commons, and differentially expressed miRNA and mRNA among NPM1 mutated (n = 21) and NPM1 wild-type (n = 162) cases were identified to establish a dynamic association at the molecular level. In vitro experiments using high-throughput RNA sequencing were performed on human AML cells carrying NPM1 mutated and wild-type allele. The comparison of in vitro transcriptomics data with in silico miRNA–mRNA expression network data revealed downregulation of SMC1A. On establishing miRNA–mRNA interactive pairs, it has been observed that hsa-mir-215-5p (logFC: 0.957; p = 0.0189) is involved in the downregulation of SMC1A (logFC: –0.481; p = 0.0464) in NPM1 mutated AML. We demonstrated that transient expression of NPM1 mutation upregulates miR-215-5p, which results in downregulation of SMC1A. We have also shown using a rescue experiment that neutralizing miR-215-5p reverses the effect of NPM1 mutation on SMC1A. Using the leukemic blasts from AML patients, we observed higher expression of miR-215-5p and lower expression of SMC1A in NPM1 mutated patients compared to wild-type cases. The overall survival of AML patients was significantly inferior in SMC1A high expressers compared to low expressers (20.3% vs. 58.5%, p = 0.018). The data suggest that dynamic miR-215-SMC1A regulation is potentially modulated by NPM1 mutation, which might serve as an explanation for the better outcome in NPM1 mutated AML.

Key words: miRNA–mRNA; NPM1 mutation; Acute myeloid leukemia (AML); SMC1A

INTRODUCTION

Acute myeloid leukemia (AML) is characterized by uncontrolled clonal proliferation of poorly differentiated cells of the myeloid lineage. Although more than 40 mutations in nucleophosmin (NPM1) have been reported, the most common mutations are characterized by tetranucleotide insertion in exon 12 accounting for 45%–60% of AML patients with normal karyotype (NK). Although it is well established that NPM1 mutation is an AML-driving lesion with good prognostic impact, the rationale behind this prognostication is not well defined. Some studies suggested that NPM1 mutation induces chemosensitivity in leukemic cells, but the precise cause is yet to be elucidated. Mutant NPM1 AML has a unique microRNA (miRNA) and their target gene (mRNA) signature compared to wild-type NPM1.

In humans, most of the proteins coding RNAs are subject to miRNA–mRNA-mediated regulation. To reliably measure such interactions, prior knowledge of target mRNAs is required. However, with the advent of next-generation sequencing methods, it has now become possible to investigate these interactions globally. Dynamic regulation of miRNA–mRNA has been reported to...
influence the prognostic outcome in glioblastoma and breast cancer. Becker et al. described the gene expression signature of a series of older patients with NK-AML and NPM1 mutations that featured upregulation of homeobox genes accompanied by higher expression levels of miR-10a, miR-10b, miR-196a, and miR-196b. The miRNA–mRNA interaction to influence the prognosis in NPM1 mutated AML needs to be investigated. A recent study suggested that the molecular interaction of SMC1A with miRNA and long noncoding RNA could be used as a promising therapeutic target to mitigate the progression of AML. The SMC1A has also been shown to promote cell proliferation, migration, and metastasis in various solid tumors. The SMC1A belongs to the cohesin complex family of genes contributing toward chromosomal segregation. Recurrent mutation of SMC1A and the other members of cohesin complex genes affects the prognosis in AML. It has also been suggested that genetic alterations in SMC1A may contribute in leukemiogenesis through premature sister chromatid separation.

In this study, we aim to evaluate the pairwise correlations of differential expression between miRNA and mRNA, and if the strength of differential (negative or positive) regulation of an miRNA on its target gene can be modulated by NPM1 mutation in AML. The miRNA and mRNA expression data of AML patients from genome data commons (GDC) was analyzed for miRNA–mRNA interactions that were subsequently divided into two groups (NPM1 mutated and wild type). This in silico data were validated in vitro using human AML cell line OCI-AML3 (NPM1mutwild-type-mimic) and the OCI-AML3-siNPM1mut cells (NPM1mutWild-Type-Mimic) using high-throughput RNA sequencing. The findings from in silico and in vitro analyses were further validated using leukemia cell lines and biological material from an independent cohort of AML patients. We observed that miR-215-5p mediates the downregulation of SMC1A in the presence of NPM1 mutation.

MATERIALS AND METHODS

Transcriptomics Analysis From GDC: In Silico Analyses

The miRNA, mRNA (genes), and clinical information were obtained from TCGA (The Cancer Genome Atlas) and TARGET (Therapeutically Applicable Research to Generate Effective Treatments) database of GDC portal. A total of 988 and 200 AML cases from TARGET and TCGA, respectively, were identified. One hundred ninety-one cases were selected in which miRNA and mRNA expression data were available along with clinical information. Based on available clinicobiological data, cases were divided into NPM1 mutated (n = 21) and NPM1 wild-type (n = 162) groups. For 183 cases, high-throughput sequencing (HT-seq) count files of miRNA and mRNA were obtained and differentially expressed miRNA and mRNA with p value and false discovery rate (FDR) were identified using EdgeR Bioconductor package v3.20.9. All the data derived from GDC are referred as in silico analyses.

High-Throughput Transcriptional Profiling (RNA Sequencing): In vitro Analyses Knockdown of NPM1 Mutation in OCI-AML3 Cells

Human AML cell line OCI-AML3 (NPM1mut) carrying endogenous heterozygous NPM1 type A mutation and the OCI-AML3-siNPM1mut cells (NPM1mutWild-type-mimic) were used for in vitro validation tests for miRNA and mRNA expression profiling. The mutated allele of NPM1 in OCI-AML3 cells was knocked down by reverse transfection using the small interfering RNA (siRNA) (GE Healthcare Dharmaco, Lafayette, CO, USA) that specifically targets the mutated NPM1 allele but not the wild-type allele to generate OCI-AML3-siNPM1mut cells. Sequences of the siRNAs are provided in Supplementary Table 1a (available at https://drive.google.com/open?id=12EmifB1BFJCwG6-Cp39kQhPnlxHLDh).

Evaluation of NPM1 Mutation Knockdown Efficiency

Following reverse transfection, RNA was isolated from NPM1mutWild-type-mimic and NPM1mutScramble siRNA cells using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA), complementary DNA (cDNA) synthesis (Thermo Fisher Scientific), and polymerase chain reaction (PCR) using LightCycler 96 Real-Time PCR System (Roche Diagnostics GmbH, Mannheim, DEU) with previously described primers and probes for NPM1 mutation quantification. Abelson (ABL) gene was used as an endogenous control to assess the quality of CDNA. Cell lystate preparation and Western blot analyses were performed according to standard procedures. The mouse monoclonal antibody recognizing specifically NPM1mutScramble (#PA1-46356; Thermo Fisher Scientific) was used. The monoclonal β-actin antibody (#A5316; Sigma-Aldrich, St. Louis, MO, USA) was used as a control.

RNA sequencing of NPM1mutScramble and NPM1mutWild-Type-Mimic

Total RNA was isolated from NPM1mutWild-type-mimic and NPM1mutScramble cells using TRIzol reagent (Thermo Fisher Scientific). Trilink CleanTag Small RNA kit (Trilink Biotechnologies, San Diego, CA, USA) was used to prepare small RNA library. The small RNA is first captured using specific RNA adapters that ligate to the 3′ and 5′ ends of the small RNA. These adapters are each about 60 bases long, and they ligate to the small RNA, which is around 18–24 base pairs (bp) long. These adapter-ligated fragments (around 140 bases long) of
small RNA are then reverse transcribed, converting them to cDNA. The small RNA converted to adapter-ligated cDNA fragments were then amplified using PCR. The small RNA libraries thus prepared were checked on tapestation and sequenced on Illumina HiSeq 2500 in 1 × 50 single end run.

Data Analysis Pipeline. For mRNA-seq, quality of the fastq files was checked using FastQC for base and sequence quality score distribution. The paired-end reads were aligned to reference human genome Hg19 using genes and transcript (GTF) file from Ensembl by running the STAR 2.4.1 program. The aligned reads were used for estimating the expression of the genes and transcripts using cufflinks-2.2.1. The expression values are reported in FPKM (fragment per kilo per million) units for each of the genes and transcripts. The differential expression analysis is performed using DESeq2 and is reported as log, fold change.

Similarly, for miRNA-seq, quality of the fastq files was checked using FastQC for base and sequence quality score distribution. The reads were aligned to the reference human genome (Hg19) and miRBase database using Bowtie 2. The aligned reads were used for estimating the expression of the miRNA using miRCat UEA Small RNA Workbench v3.2.

Independent Validation of NPM1 Mutation Effect on miR-215 and SMC1A Expression

Cell lines, Plasmids, and Transfection. Human myelogenous leukemia cell line K562 (generously provided by Professor Pelicci, IFOM Milan, Italy) was used for in vitro validation. The plasmids (pEGFP-C1) containing NPM1 mutant and NPM1 wild-type cDNA insert (provided by Prof. B. Falini, Perugia, Italy) were electroporated into K562 cells using Gene Pulser Xcell™ Electroporation Systems (Bio-Rad, Hercules, CA, USA), and cells were labeled as K562[TRL] (only cells), K562[NPM1-wt] (plasmid with wild-type NPM1), and K562[NPM1-mut] (plasmid with NPM1 mutant type A). Following electroporation, GFP-positive cells were sorted using FACSAria III (BD, Franklin Lakes, NJ, USA). RNA from sorted cells was isolated, and mutant NPM1 was confirmed by Sanger sequencing. Total RNA and miRNA were extracted from K562[TRL], K562[NPM1-wt], and K562[NPM1-mut] cells using miRNeasy Mini kit (Qiagen, Venlo, Netherlands) as per the manufacturer’s instructions. RQ-PCR analysis of miR-215-5p was carried out using the miRCURY LNA PCR Kit (Qiagen, Venlo, Netherlands). The miRNA expression analysis was performed using SYBR green-based amplification using light-designed primers (Supplementary Table 1a; available at https://drive.google.com/open?id=12EmifBIBFJcwbG6-Cp39kQhPnlgxHLDdb). The template variation across samples was normalized using endogenous controls of miR-103-3p and β-actin for miRNA and mRNA, respectively. The comparative threshold cycle (2^ΔΔCT) method was used to analyze the data.

Inhibition of miR-215-5p Resulted in Upregulation of SMC1A. The miR-215-5p inhibitor (Cat. No. MIH01561; ABM Canada) was used to rescue the expression of SMC1A in OCI-AML3 cells, which carries an endogenous NPM1 mutation type A. A total of 0.5 × 10^6 OCI-AML3 cells (in duplicates) were transfected with 0.1 and 0.5 µg of miR-215-5p inhibitor and incubated at 37°C for 48 h as per the manufacturer’s instruction. Following incubation, total RNA was isolated using TRIzol method from inhibitor-treated and control cells. The cDNA for miR-215-5p and SMC1A expression was synthesized and quantified by real-time PCR in triplicates.

Analysis of the Prognostic Impact of SMC1A Expression in AML: Patient Samples, cDNA Synthesis, and RQ-PCR

To study the impact of SMC1A expression, survival analysis was performed in an independent cohort of AML patients. Bone marrow samples from 67 patients (NPM1 wild type n = 49, and NPM1 mutated n = 18) at the time of diagnosis were collected. The median age of the cohort was 27 years (range: 15–58 years). Out of 67 patients, 4 patients died during induction, 5 were refractory to the treatment, while the follow-up data were not available for 2 patient, and hence these cases were excluded from SMC1A expression and overall survival (OS) analysis. All patients provided written informed consent in accordance with the Declaration of Helsinki, and the Ethics Committee of Tata Memorial Centre (TMC), Mumbai, approved the study (DCGI registration number: IECIII: ECR/149/Inst/MH/2013/RR/2016 approved the study; approval reference number: 179/2015). Total RNA from AML blasts (1 µg) was used for cDNA synthesis using high-capacity cDNA synthesis kit (Applied Biosystems, Foster City, CA, USA). The SYBR green-based RQ-PCR assays for SMC1A expression were carried out using LightCycler 96 real-time PCR system. According to the median values of SMC1A/ABL1 expression, AML patients were divided into high expressers and low expressers. Results of SMC1A/ABL1 expression data were analyzed for their impact on OS. In addition to AML patients recruited at TMH (n = 56), we also included SMC1A expression data of AML patients (n = 179) from GDC for OS analyses.

Statistical analysis was performed using IBM SPSS v21. OS was defined as the time from the start of induction therapy to time of last follow-up or death. Results of the SMC1A and miR-215-5p expression were analyzed for their impact on OS using the Kaplan–Meier method. Quantitative variables were analyzed by Student’s t-test. All statistical analysis was two sided, and a value of p < 0.05 was considered statistically significant.
RESULTS

miRNA and mRNA Expression of NPM1 Wild-Type and Mutated Patients: In Silico Data

To understand the effect of NPM1 mutation on miRNA–mRNA network, public domain gene expression repository was used to correlate with functional assay derived from NPM1mutscramble and NPM1mutwild-type-mimic. Out of 200 TCGA AML cases, miRNA-seq and mRNA-seq data were available for 103 and 151 AML cases, respectively. Concerning TARGET database (N = 988), 265 miRNA-seq and 179 RNA-seq were reported. To study the miRNA–mRNA interaction within the same patients, 85 cases were identified from TCGA and 106 cases from TARGET having miRNA and mRNA expression along with clinicobiological information. The read counts data for miRNA expression consist of 1,881 miRNA, while mRNA expression data consisting of 55,640 mRNA were extracted from 191 cases derived from TCGA and TARGET. In clinicobiological files of 191 cases, NPM1 mutation status was not reported for 8 cases. Hence, these cases were excluded from the analyses. The differentially expressed miRNA and mRNA are reported in Supplementary Tables 1b and 1c, respectively (available at https://drive.google.com/open?id=12EmifBlBFJCwbG6-Cp39kQhPnlgxHLDb).

Identification of Genes Targeted by miRNAs Associated With Mutated NPM1: miRNA–mRNA Interactive Pairs

The differential miRNA expression between two groups (NPM1 wild-type and mutant AML) has been reported in Table 2. With respect to GDC dataset, the list of miRNAs was generated based on the difference in the miRNA expression (log2 fold change) between the two groups with a value of \( p < 0.05 \). The log2 fold change was used to categorize the miRNAs in NPM1 wild-type and mutant groups. Two miRNAs (hsa-mir-7976 and hsa-mir-6718) were downregulated, while four (hsa-mir-215, hsa-mir-450a-1, hsa-mir-5087, and hsa-mir-3912) were upregulated in NPM1mutated AML compared to NPM1mutwild-type-mimic. Based on the cutoff of 1.5 log fold change, 27 miRNA were upregulated while 33 were downregulated. Similarly, differently expressed mRNA revealed 2,983 upregulated and 807 downregulated genes as a result of NPM1 mutant allele silencing.

Knockdown of NPM1 Mutant Allele and RNA Sequencing

RQ-PCR using plasmid calibration curve has allowed accurate assessment of NPM1 mutation A copies/10⁴ABL1 in OCI-AML3 versus OCI-AML3-siNPM1mut cells. We observed 73.5% of reduction of NPM1 mutation A expression in siRNA-mediated knockdown cells (Table 1). Western blot data revealed 64% repression efficiency of mutated NPM1 (Fig. 1). The data were analyzed using ImageJ software29. In vitro differentially expressed miRNA and mRNA are reported in Supplementary Table 1d and 1e, respectively (available at https://drive.google.com/open?id=12EmifBlBFJCwbG6-Cp39kQhPnlgxHLDb) in NPM1mutscramble compared to NPM1mutwild-type-mimic. Based on the cutoff of 1.5 log fold change, 27 miRNA were upregulated while 33 were downregulated. Similarly, differently expressed mRNA revealed 2,983 upregulated and 807 downregulated genes as a result of NPM1 mutant allele silencing.

Table 1. Knockdown Efficiency of NPM1 Mutation A Using Specific siRNA

| Sample                     | NPM1 Mutation A/10⁴ABL1 Copy Number | % of NPM1 Mutation A Expression (siRNA-Treated Cells ×100/Scramble-Treated Cells) | % of NPM1 Mutation A Knockdown |
|----------------------------|-------------------------------------|---------------------------------------------------------------------------------|--------------------------------|
| Treated cells (siRNA 60 nM)| 12,000                              | 26.7                                                                             | 73.3                           |
| Treated cells (scramble siRNA 60 nM) | 45,000                              |                                                                                  |                                |
To obtain the target genes of 14 miRNAs associated with NPM1 mutation, the miRTar database (http://mirtar.mbc.nctu.edu.tw/human/) was used. The miRTar database also gives the mature miRNA, which is known to form a pair with the target genes. Given the fact that one miRNA can regulate the expression of multiple genes, we focused miRNA interactive analyses on the genes that are known to be modulated in leukemia. The comprehensive panel of 54 genes of Illumina’s TruSight myeloid leukemia was used. These genes were searched in the list of target genes associated with 14 miRNAs from the miRTar database. Out of 14 miRNAs, 5 miRNAs (hsa-miR-363, hsa-mir-607, hsa-mir-382, hsa-mir-215, and hsa-mir-449a) form pair with 13 genes from TruSight Myeloid Sequencing panel. The differential expression of 13 genes and their corresponding miRNAs has been reported in Table 3.

On establishing miRNA–mRNA interactive pairs, it was observed that hsa-mir-215-5p (LogFC: 0.9579 and p-value: 0.0189, Table 2) is involved in the downregulation of SMC1A (LogFC: −0.4816 and p = 0.0464) in NPM1 mutated AML.

**Effect of NPM1mut on miR-215-5p and SMC1A Expression**

Expression of NPM1 type A mutation in K562 cells was verified using Sanger sequencing (Fig. 2a). K562 NPM1-mut cells showed significantly higher expression of miR-215-5p compared to K562 CTRL cells. The upregulation of miR-215-5p was approximately 1.45-fold in K562 NPM1-mut cells in comparison to K562 CTRL cells (p = 0.0050) (Fig. 2b). We also observed significant downregulation of SMC1A expression in K562 NPM1-mut cells compared to NPM1wild-type cells (p = 0.0010) and NPM1 CTRL cells (p = 0.0029) (Fig. 2c). We further checked the effect of miR-215-5p inhibition on SMC1A expression in OCI-AML3 cells comprising endogenous NPM1 type A mutation. Compared to control, we have shown 1.85- and 2.66-fold reduction in the levels of miR-215-5p after 0.1 and 0.5 µg of inhibitor treatment, respectively (Fig. 3a). On analyzing the effect of miR-215-5p inhibition on the expression of SMC1A, we observed 1.20- and 1.90-fold increase in SMC1A transcript levels (Fig. 3b).

**SMC1A and miR-215-5p Expression in AML Patients: Survival Analyses**

Among 65 analyzed cases, the mean SMC1A expression value in NPM1 wild type (n = 47) was 2.99 (range: 0.56–20.33), while in NPM1 mutated patients (n = 18), the mean value was 1.14 (range: 0.08–3.79). We observed significantly lower expression of SMC1A in NPM1 mutated patients compared to NPM1 wild-type (p = 0.0018) (Fig. 4a). We carried out expression study

| miRNA | Mature miRNA | Genes (mRNA) | Log2 FC | p Value |
|-------|--------------|--------------|---------|---------|
| hsa-miR-363 | hsa-miR-363-3p | NRAS | 0.0614 | 0.7581 |
| hsa-mir-607 | hsa-mir-607 | NPM1 | 0.2240 | 0.1686 |
| hsa-mir-382 | hsa-mir-382-5p | PTEN | 0.1741 | 0.3946 |
| hsa-mir-215 | hsa-mir-215-5p | SMC1A | 0.1821 | 0.0984 |
| hsa-mir-215-3p | hsa-mir-215-5p | BCR | 0.1641 | 0.0679 |
| hsa-mir-449a | hsa-mir-449a | NOTCH1 | 0.1501 | 0.0845 |

**Table 2. miRNA Expression in Acute Myeloid Leukemia (AML) Patients With NPM1 Mutation Compared to NPM1 Wild-Type AML**

| miRNA   | Log2 FC | p Value | FDR  |
|---------|---------|---------|------|
| hsa-mir-363 | 1.9336  | 0.0002  | 0.0105 |
| hsa-mir-1291 | −1.3753 | 0.0004  | 0.0176 |
| hsa-mir-607 | 2.3239  | 0.0014  | 0.0405 |
| hsa-mir-7976 | −1.1299 | 0.0020  | 0.0541 |
| hsa-mir-382 | −1.9266 | 0.0027  | 0.0623 |
| hsa-mir-3912 | 1.0942  | 0.0031  | 0.0677 |
| hsa-mir-6718 | −1.2294 | 0.0045  | 0.0841 |
| hsa-mir-3911 | 2.7209  | 0.0047  | 0.0851 |
| hsa-mir-5087 | 1.8014  | 0.0081  | 0.1154 |
| hsa-mir-4786 | 1.0669  | 0.0088  | 0.1203 |
| hsa-mir-450a-1 | 1.4821  | 0.0134  | 0.1545 |
| hsa-mir-215 | 0.9579  | 0.0189  | 0.2443 |
| hsa-mir-6747 | 1.2568  | 0.0339  | 0.3393 |

FC, fold change; FDR, false discovery rate.
Figure 2. (a) Induced expression of NPM1 type A mutation in K562 leukemia cells. (b) Expression of NPM1 type A mutation modulated expression of miR-215 and (c) SMC1A in K562CTRL, K562NPM1-wt, and K562NPM1-mut cells. ΔCt = difference in threshold cycles of SMC1A with respect to ABL1 (endogenous control). *p ≤ 0.05, **p ≤ 0.01.

Figure 3. (a) Quantitative real-time polymerase chain reaction (PCR) expression of miR-215-5p and (b) SMC1A in OCI-AML3 cells treated with miR-215-5p inhibitor. ΔCt = difference in threshold cycles of SMC1A with respect to ABL1 and miR-103-3p, respectively (endogenous control). *p ≤ 0.05, ****p ≤ 0.0001.

Figure 4. (a) Quantitative real-time PCR expression of SMC1A and (b) miR-215-5p in AML patients accrued at Tata Memorial Centre. ΔCt = difference in threshold cycles of SMC1A and miR-215-5p with respect to ABL1 and miR-103-3p, respectively (endogenous control). *p ≤ 0.05, **p ≤ 0.01.
of miR-215-5p in 26 patients (NPM1 wild-type $n = 12$ and NPM1 mutated $n = 14$) for which biological material was available. The mean miR-215-5p expression value in NPM1 wild-type was 5.78 (range: 3.47–8.54), while in NPM1 mutated patients, the mean value was 7.51 (range: 4.19–11.07). We observed significantly higher expression of miR-215-5p in NPM1 mutated patients compared to NPM1 wild-type ($p = 0.03$) (Fig. 4b).

For the purposes of clinical correlation, we examined the utility of high SMC1A/ABL1 expression as a prognostic marker to predict survival outcome in AML. The survival data derived from TMC cohort ($n = 56$) have shown that low SMC1A/ABL1 expressers were doing better compared to high expressers ($p = 0.018$ at 3 years $58.5\%$ vs. $20.3\%$) (Fig. 5a). The results were corroborated with the AML patients from GDC ($n = 179$), where OS of high SMC1A/ABL1 expressers was inferior compared with low SMC1A/ABL1 expressers ($p < 0.0001$ at 6 years $23.6\%$ vs. $53.6\%$) (Fig. 5b). Similar findings were observed when data from GDC and TMC cohorts pooled together to assess the survival benefits in SMC1A low expressers (Fig. 5c). With respect to hsa-miR-215 expression and survival analyses, we observed superior OS of high hsa-miR-215 expressers compared with low expressers, but the difference was not statistically significant (Fig. 5d).

**DISCUSSION**

The dynamic miRNA–mRNA regulation in AML is potentially modulated by NPM1 mutation. In this study, the differential miRNA–mRNA expression data in AML patients with and without NPM1 mutation were derived from an in silico approach. Subsequently,
in vitro experiments using high-throughput RNA sequencing were performed on OCI-AML3 and OCI-AML3-siNPM1mut cells. The comparison of in vitro transcriptomics data with in silico miRNA–mRNA expression network data revealed that hsa-miR-215-5p downregulates SMC1A expression in the presence of NPM1 mutation. Furthermore, bioinformatic data including expression analyses also revealed that NPM1 mutation status plays a major role in altering the expression of SMC1A and miR-215-5p, which was shown in K562 cells and using AML patients’ samples (Figs. 2 and 4). We have also shown using a rescue experiment neutralizing miR-215-5p reverse the effect of NPM1 mutation on SMC1A. The SMC1A expression in AML is reported to influence event-free survival and OS31; however, the limited number of AML cases precludes to draw any conclusion. The impact of SMC1A expression on survival was analyzed in AML patients from GDC portal as well as an independent cohort of AML patients who were recruited at our center. Findings obtained from both the analyses have confirmed the low SMC1A expression is associated with an improved outcome in AML (Fig. 5).

On analyzing quantitative expression data of SMC1A in NPM1 wild-type and mutated AML patients, we observed significantly high SMC1A expression in wild-type patients (p = 0.0018) (Fig. 4A). However, we could not perform survival analyses based on NPM1 mutation status due to the limited number of NPM1 positive cases in our cohort. The SMC1A belongs to the family of cohesin complex, which comprises SMC1A, SMC3, RAD21, STAG2, and STAG1. These proteins form a ring structure that regulates chromosome segregation. Therefore, cohesin complex is an essential structure during cell division. AML blasts are known to have increased proliferation potential due to unregulated cell division. The mutations of the genes in cohesin complex have been described in 13% of AML patients32. Thol and colleagues have shown a strong correlation between the mutated cohesion gene and NPM1 mutations (57% of cohesin gene mutated patients had an NPM1 mutation)33. Recently, Patel et al. also demonstrated that most of the NPM1-mutated AML patients harbor concurrent mutations in genes involved in the regulation of DNA methylation, RNA splicing, and the cohesin complex33. On analyzing the functional effect of SMC1A mutations, Mannini et al. observed that the mutations of SMC1A do not affect SMC1A expression34. However, it has been proposed that altered SMC1A can influence the dynamic relationship between the cohesin complex and chromatin, leading to altered expression of proto-oncogenes or tumor suppressor genes. It has been demonstrated that SMC1A overexpression is associated with colorectal cancer (CRC) development and can be used as a potential therapeutic target in CRC16,35.

Several different miRNAs have been associated with NPM1 mutated AML; however, strong upregulation of miR-10a-5p and miR-10b-5p has been frequently reported6,36. Garzon and colleagues reported that AML with NPM1 mutation shows distinct miRNA, mRNA, and miRNA–mRNA signatures6. Chiu et al. recently identified nine miRNA–mRNA regulatory pairs that could predict patients’ outcome in NPM1 mutated AML37.

To the best of our knowledge, no study has shown the miR-215-5p association with NPM1 mutation. Within AML, it has been reported that miR-215-5p is significantly downregulated in FLT3-ITD mutated AML cases. However, it is yet to be established that miR-215-5p adversely affects the survival independent of other biomarkers in AML.

CONCLUSION

The downregulation of SMC1A mediated by miR-215-5p suggests its role behind the better prognosis of NPM1 mutated AML compared to AML with wild-type NPM1. Considering the small cohort of patients in the present study, future studies are warranted to confirm these findings in a larger cohort of AML patients.

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