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Original Article

MiR-659-3p regulates the progression of chronic myeloid leukemia by targeting SPHK1

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Abstract: Accumulating evidence shows that microRNAs (miRNAs) are significant regulators of multiple cellular processes including chronic myelocytic leukemia (CML). However, the mechanism of miR-659-3p in CML remains unclear. In this study, we aimed to investigate the potential role of miR-659-3p in CML progression. We found that miR-659-3p was down-regulated in CML. The upregulation of miR-659-3p significantly suppressed the proliferation ability, and enhanced the apoptosis capacity of K562 cells. Simultaneously, we found that sphingosine kinase 1 (SPHK1) was up-regulated in CML. MiR-659-3p performed its function through downregulating SPHK1 by binding to its untranslated region (3'-UTR). These results suggested that miR-659-3p, acted as a tumor suppressor, decreased the proliferation ability of K562 cells, and increased the apoptosis capacity of K562 cells. Therefore, our study provided a new theoretical basis of miR-659-3p which may be a promising approach for CML treatment.

Keywords: miR-659-3p, SPHK1, chronic myeloid leukemia

Introduction

Chronic myeloid leukemia (CML) is a common malignant hematopoietic disorder with the accumulation of a large number of immature white blood cells in the bone marrow and peripheral blood [1-3]. It is characterized cytogenetically by the presence of the Philadelphia (Ph) chromosome and is consistently associated with a reciprocal translocation t [9; 22] [q34; q11] [4]. Approximately 90% of patients with CML have this acquired genetic abnormality. An abnormal Breakpoint cluster region/Abelson oncogene (BCR/ABL) fusion gene which encodes a 210-Kd (P210) fusion protein with prominent tyrosine kinase activity and transforming ability is generated during the translocation [5]. The tyrosine kinase inhibitor (TKI), matinib mesylate is currently used to treat of CML [6, 7]. However, it is effective in controlling but not curing the disease. In addition, some side-effects including diarrhea and allergic reaction remain serious clinical problems [8]. Therefore, a better understanding of the molecular pathogenesis of CML and therapeutic avenues are extremely urgent.

MicroRNAs (miRNAs) are a class of short endogenous non-coding RNAs with 20-25 nucleotides in length. IThey participate in post-transcriptional regulation by binding to the 3'-untranslational region (3'-UTR) of target mRNAs [9] and play important roles in the biological processes of cell proliferation, differentiation, apoptosis, and invasion [10-13]. It has been confirmed that miRNAs can function as tumor suppressors or oncogenes in various cancers including CML. Much research has shown that abnormal miRNA expression may induce the occurrence of CML [14, 15]. Quan Li et al suggested that miR-130a may function as a novel tumor suppressor in CML by targeting and inhibition of RECK directly [14]. Peng Yang et al demonstrated that miR-362-5p was overexpressed in cells and clinical specimens of CML by down-regulating Growth arrest and DNA damage-inducible (GADD) 45α [16]. However, the functional relevance of miR-659-3p in carcinogenesis and progression of CML remains unclear. We used the prediction algorithms of TargetScan to determine the target genes that mediate the effects of miR-659-3p in CML and selected SPHK1, which has a conservative

miR-659-3p binding site in its 3'UTR with high specificity and is a conservative enzyme that regulates the balance between ceramide/sphingosine and sphingosine-1-phosphate (S1P) levels [14]. It has been demonstrated that SPHK1 mediated cellular behaviors plays a critical role in the regulation of cancer cell survival, proliferation and transformation [15-17]. However, it is not well documented whether SPHK1 is responsible for malignant transformation of CML.

Therefore, in this study, we provided evidence that miR-659-3p expression was significantly decreased in CML. It might function as a tumor suppressor through regulating SPHK1 expression in CML. These results indicated that SPHK1 expression was negatively correlated with the expression level of miR-659-3p in CML, and miR-659-3p down-regulated the expression of SPHK1 by directly targeting the 3'UTR of SPHK1 mRNA. We demonstrated that miR-659-3p inhibited cell proliferation, and promoted cell apoptosis by downregulating the expression of SPHK1. Therefore, miR-659-3p may be a potential biomarker for the detection and treatment of CML.

Materials and methods

Clinical specimens

Sixty-eight patients with CML and sixty-eight healthy donors were included in this study. Patients with CML were recruited from West China Hospital, Sichuan University. Healthy volunteers were recruited from the Sichuan University. The study was approved by Ethics committees of the West China Hospital, Sichuan University. Informed consent was signed by the participants.

Cell lines and transfection

K562 cell lines were purchased from College of Life Science, Hunan Normal University, China. The K562 cells were cultured in RPMI-1640 (Invitrogen, Carlsbad, CA, USA) including 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), penicillin (100 U/ml), and streptomycin (100 μg/ml) at 37°C and 5% CO_2. For the treatment, 2×10^5 K562 cells were cultured in 6-well plates and then transfected with 200 μl mature miR-659-3p mimic, or inhibitor (GenePharma Co., Ltd., Shanghai, China) for 72 hrs. All transfections were completed using Lipofectamine™ 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols.

Quantitative polymerase chain reaction (qPCR) assay

Total RNA was extracted from the clinical specimens and the treated K562 cells according to the manufacturer's instructions using TRIzol (Invitrogen, Carlsbad, CA, USA) A miScript II RT Kit (Qiagen, Hilden, Germany) was used to reverse transcribe RNA into cDNA and the mRNA expression levels were detected using the SYBR-Green PCR Master Mix kit (Takara). Gene mRNA expression was normalized to β-actin. The target genes and controls were analyzed by qRT-PCR and the reactions were performed on ABI 7500 system (Applied Biosystems, Carlsbad, CA, USA) with primers specific for miR-659-3p (Qiagen, Hilden, Germany). The primer sequences for SPHK1 are: 5'-CAC TGA GCG GCG GAA CCA C-3' (forward primer) and 5'-GCT GGA CCA CAA CGG GGG A-3' (reverse primer); The primer sequences for GAPDH are: 5'-TGT TCG TCA TGG GTG TGA AC-3' (forward primer) and 5'-ATG GCA TGG ACT GTG GTC AT-3' (reverse primer); The primer sequences for miR-659-3p are: 5'-ACT GCT CGA GCA CTG TCA TTA TTT TCT CAC-3' (forward primer) and 5'-ACT GAG ATC TGC GTT CTT GTT TTG TGT TTC-3' (reverse primer); The primer sequences for U6 are: 5'-CTC GCT TCG GCA GCA CA-3' (forward primer) and 5'-AAC GCT TCA CGA ATT TGC GT-3' (reverse primer). Samples were normalized to GAPDH. All data are performanced as the mean ± SD of three independent experiments.

Western blot analysis

Total protein extracted from the treated K562 cells was separated by SDS-PAGE gels based on the molecular weight of the objective proteins. GAPDH was used as a loading control. The protein in gels was transferred to a PVDF membrane (PerkinElmer, Boston, MA). The membranes were blocked in TBST (Tris-buffered saline with 0.5% Triton X-100) containing 5% nonfat milk and probed with primary antibodies against SPHK1 (Abcam, Cambridge, MA, USA) and GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C. The membranes were subsequently washed with 0.1 M

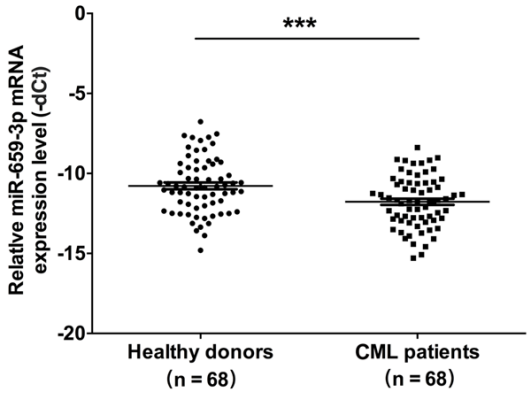

Figure 1. Expression levels of miR-659-3p in CML patients and healthy donors. The expression levels of miR-659-3p were measured by quantitative real-time PCR (qRT-PCR). The Figure shows that miR-659-3p was significantly decreased in CML patients compared with healthy donors. Data are presented as the mean ± SD of 3 experiments. ***$P < 0.05$ versus the healthy donors group.

phosphate buffered saline (PBS) with Tween-20 and then incubated with HRP-conjugated secondary antibodies. Signals were detected using the enhanced chemiluminescence western blotting system (ComWin Biotech, Beijing, China).

Luciferase reporter assay

K562 cells were cultured in a 24-well plate, cotransfected with miR-659-3p mimic, mimic negative control, miR-659-3p inhibitor or inhibitor negative control and WT or Mut 3'-UTR (Whole gene synthesis and then insertion into pGL3, Genema, Shanghai, China) of SPHK1. Cells were collected and washed using PBS and lysed in Passive Lysis Buffer (Promega, WI, USA) at 48 hrs after transfection. The Dual-Luciferase Reporter Assay System (Promega, Wisconsin, WI, USA) was used to analyze the data. Data are shown as the means ± SD of three independent experiments.

MTT assay

Cell viability was assessed via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-trtrazolium bromide (MTT) assay. The treated cells were seeded in 6-well plates at a density of 1.5×10^5 cells per well with complete medium. When the cells adherent, 0.5 mg/mL MTT was added to each well and incubated at 37°C for 4 hrs. After that the supernatant was carefully aspirated and 100 μl of DMSO was added. The absorbance was measured at 490 nm (Thermo, MA, USA).

Flow cytometric analysis of the cell apoptosis

The treated K562 cells suspension was stained with Propidium iodide (PI). The flow cytometry results were analyzed using the FlowJo software. Cells were segmented into four types, such as the viable cells, dead cells, the early stage apoptotic cells, the late stage apoptotic cells, and the dead cells.

Statistical analysis

The data were analyzed by the Student's t-test and variance (ANOVA) using SPSS 15.0 software (SPSS, Chicago, IL, USA). All results are expressed as means ± SD. $P < 0.05$. was considered statistically significant.

Results

Expression of miR-659-3p was decreased in CML

The expression level of miR-659-3p was detected in CML patients and healthy donors by qPCR. The results indicated that the expression level of miR-659-3p was significantly lower in CML patients than in healthy donors (***$P < 0.001$) (**Figure 1**).

miR-659-3p inhibits the the proliferation abilities and promotes apoptosis in K562 cells

Relative miR-659-3p expression level was detected by qPCR. The impact of miR-659-3p expression level on cell proliferation and apoptosis was detected in K562 cells which were transfected with the miR-659-3p mimic, mimic negative control, miR-659-3p inhibitor, or inhibitor negative control. Our results found that the expression level of miR-659-3p was significantly increased in K562 cells transfected with miR-659-3p mimic negative control compared with the control group (***$P < 0.001$). The expression level of miR-659-3p was significantly decreased in K562 cells transfected with miR-659-3p inhibitor compared with the inhibitor negative control group (***$P < 0.001$) (**Figure 2A**). The proliferation ability of K562 cells was detected by MTT assay, and results showed that miR-659-3p inhibited the proliferation capacity of K562 cells (**Figure 2B**). The apoptosis

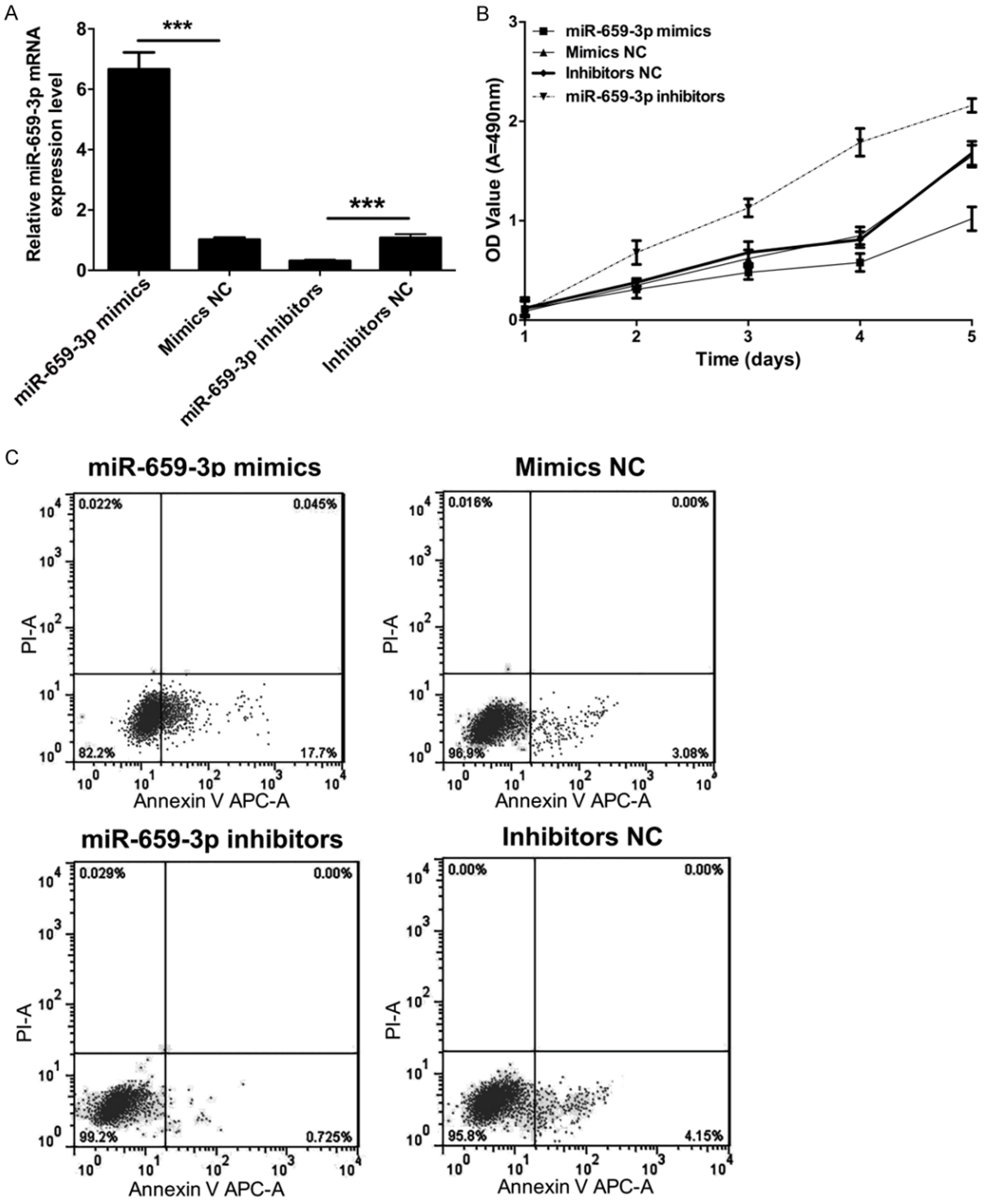

Figure 2. Effect of miR-659-3p on the proliferative ability and apoptosis capacity of K562 cells. K562 cells were transfected with the miR-659-3p mimic, mimic negative control, miR-659-3p inhibitor or inhibitor negative control. A. Relative miR-659-3p mRNA expression level was detected by qPCR. The values of expression level are represented by the mean percent of means ± SD (****P* < 0.05). B. MTT assay was performed to determine cell proliferation ability in K562 cells transfected with miR-659-3p mimic, inhibitor, or NC respectively. The values of expression level are given as mean percent of means ± SD (n = 3, ***P* < 0.05). C. K562 cells were transfected with miR-659-3p mimic, inhibitor or NC and cell apoptosis was detected by Annexin V-PI staining.

capacity was significantly decreased in K562 cells transfected with mature miR-659-3p inhibitor compared with the negative control group by Annexin V-PI staining; The apoptosis

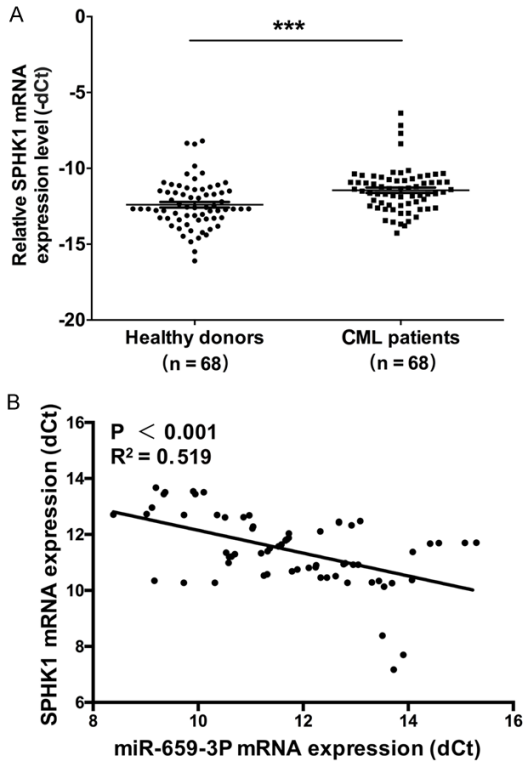

Figure 3. Expression of miR-659-3p and SPHK1 in CML patients. A. The expression of SPHK1 was measured in 68 healthy donors and 68 CML patients. B. A negative correlation between miR-659-3p and SPHK1 in 68 clinical samples was determined by Pearson's correlation coefficient (R^2 = 0.519, P < 0.001).

capacity of K562 cells transfected with miR-659-3p mimic was significantly increased compared with the negative control group (**Figure 2C**).

SPHK1 expression was negatively correlated with the expression level of miR-659-3p in CML

The mRNA expression level of SPHK1 was found to be significantly up-regulated in the CML patient group compared with healthy controls (***P < 0.001) (**Figure 3A**). The results of Pearson's correlation coefficient indicated that the SPHK1 gene was significantly negatively correlated with miR-659-3p (R^2 = 0.519, P < 0.001) in CML patients (**Figure 3B**). We hypothesize that miR-659-3p may play a role in CML by targeting SPHK1. Therefore, we performed a functional investigation in the K562 cell lines.

SPHK1 was a target gene of miR-659-3p in K562 cells

Relative mRNA expression level of SPHK1 was detected by qRT-PCR in K562 cells transfected with miR-659-3p mimic, mimic negative control, miR-659-3p inhibitor or inhibitor negative control. The results showed that the mRNA expression level of SPHK1 was significantly decreased in K562 cells transfected with mature miR-659-3p mimic compared with the control group (**P < 0.01). However, the mRNA expression level of SPHK1 was significantly increased in K562 cells transfected with miR-659-3p inhibitor compared with the control group (***P < 0.001) (**Figure 4A**). The western blotting experiment also showed a consistent result with qRT-PCR results (**Figure 4B**). SPHK1 which targeted-regulating miR-659-3p was predicted by the bioinformatic software program TargetScan. According to the results, SPHK1 has a conservative miR-659-3p binding site in its 3'UTR, and the binding to this site has high specificity (**Figure 4C**). Luciferase assay was performed in K562 cells, which were co-transfected with miR-659-3p and a luciferase reporter containing the full length of SPHK1 3'-UTR (Luc-wt) or a mutant (Luc-mut) in which the four nucleotides of the miR-659-3p-binding site were mutated. Luciferase intensity was measured after transfection for 48 hrs. miR-659-3p inhibitor markedly increased the relative fluorescence value in Luc-wt reporter constructs (***P < 0.001) (**Figure 4D**). This result confirmed that SPHK1 was a target gene of miR-659-3p in K562 cells.

miR-659-3p inhibits the progression of proliferation and promotes apoptosis of K562 cells

We further detected the effect of miR-659-3p on cell proliferation by MTT assay. Overexpression of miR-659-3p inhibited the proliferation ability of K562 cells *in vitro*, whereas re-overexpression of SPHK1 (without regulation sites) rescued this inhibition (**Figure 5A**). However, miR-659-3p inhibitors promoted the proliferation ability of K562 cells *in vitro*, whereas siSPHK1 further decreased this effect (**Figure 5B**). Overexpression of miR-659-3p increased the cell apoptosis capacity of K562 cells whereas re-overexpression of SPHK1 decreased this effect; simultaneously, miR-659-3p inhibitors decreased the apoptosis capacity of K562

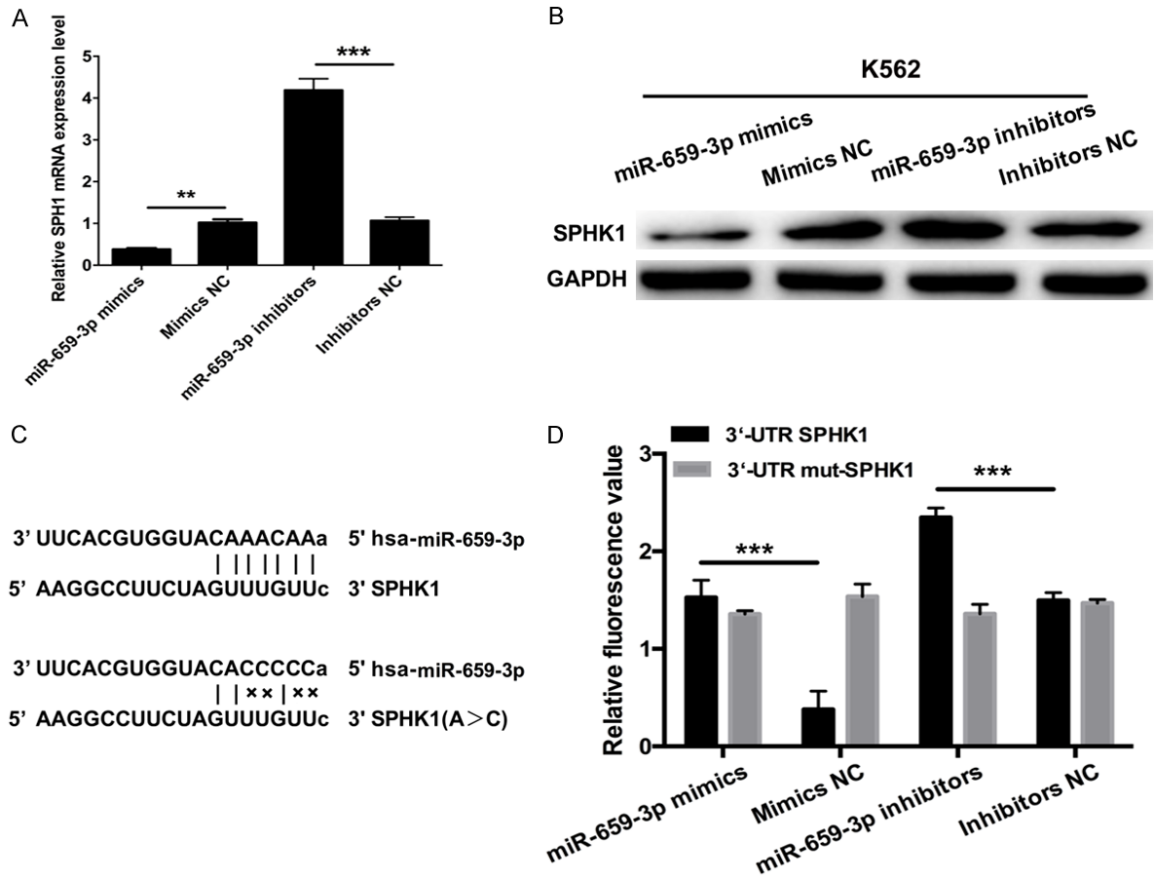

Figure 4. SPHK1 is a target gene of miR-659-3p in K562 cells. A. Relative SPHK1 mRNA expression level was detected by qPCR. B. K562 cells were transfected with miR-659-3p mimic, mimic negative control, miR-659-3p inhibitor, or inhibitor negative control, and Western blotting was used to detect the protein levels of SPHK1. GAPDH was used as control. C. WT and Mut 3'-UTR sequences of SPHK1 The wild-type and mutant SPHK1 3'-UTR contained the target sequence of miR-659-3p. D. Relative fluorescence values of SPHK1 3'-UTR (wild type and mutant constructs) were assayed after cotransfection of firefly luciferase constructs containing the SPHK1 wild-type or mutated 3'UTRs and miR-659-3p or inhibitor, oligonucleotides for 24 h in K562 cells. All of the error bars indicate means ± SD. Experiments were performed in triplicate. ***$P < 0.001$ compared with the control group.

cells, whereas siSPHK1 rescued this inhibition (**Figure 5C**).

Discussion

In recent years, numerous studies have revealed the potential roles of microRNAs as oncogenes or tumor-suppressor genes in cancer by affecting cell proliferation, migration and invasion via the regulation of different target genes [18]. At present, multiple studies have revealed that miRNA expression is a crucial regulator of CML progression, and plays an important role in CML [19-21]. A better understanding of the genes or molecular alterations is likely to be useful in early detection and future targeted treatment strategies of CML. However, the roles of dysregulated miRNAs in CML carcinogenesis remain elusive.

In this study, we provided detailed mechanistic experimental evidence of miR-659-3p in CML by suppressing the expression of SPHK1. First, we found that the expression level of miR-659-3p was down-regulated in CML patients' samples compared to controls. In addition, our observations indicated that the overexpression of miR-659-3p significantly suppressed the proliferation ability of K562 cells, and enhanced the apoptosis ability of K562 cells, whereas miR-659-3p inhibitor reversed the effects. In conclusion, we suggest that miR-659-3p acts as a novel anti-oncogenic miRNA that exerts an important effect on CML progression.

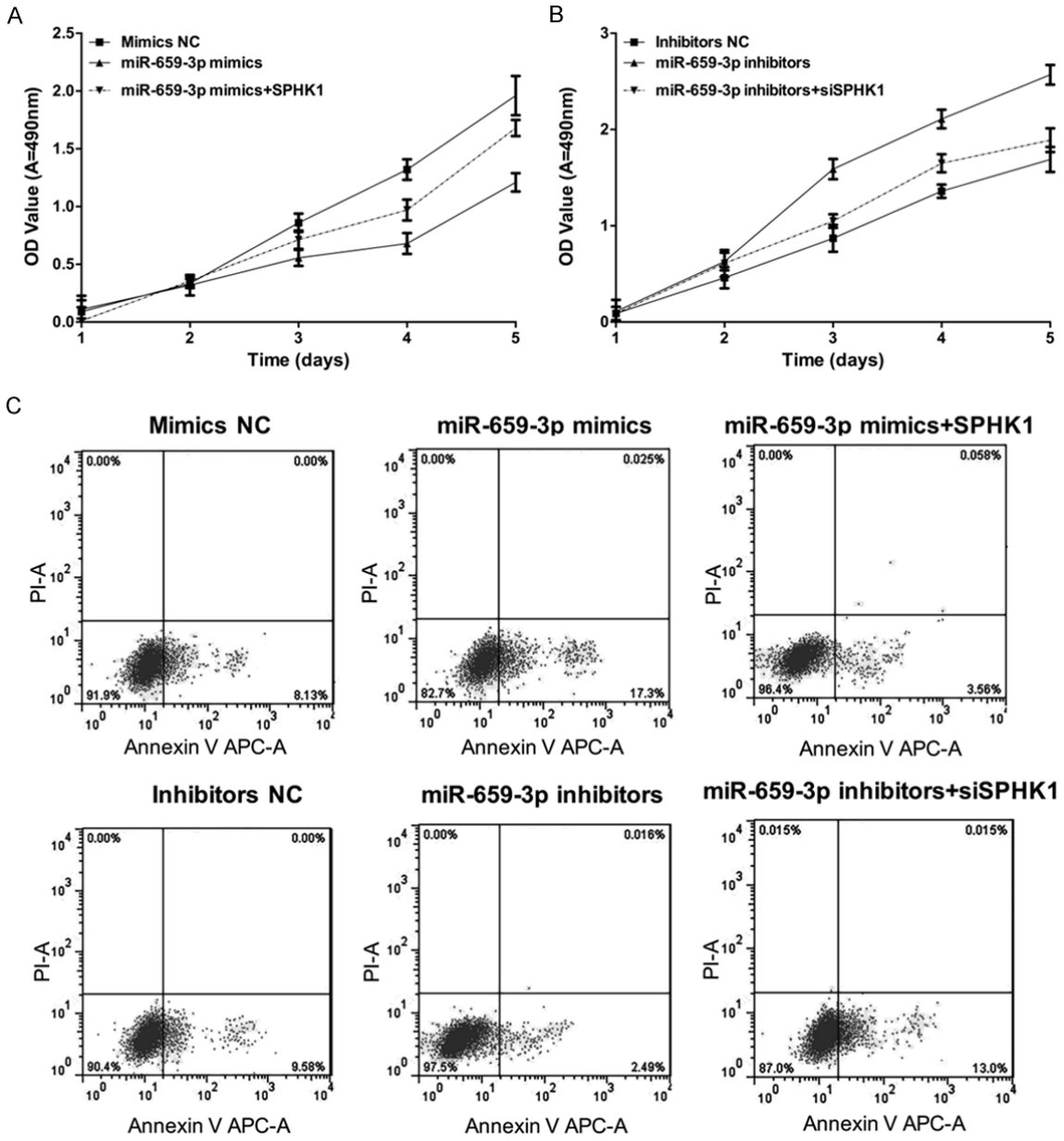

Figure 5. Effect of miR-659-3p on proliferation and apoptosis by target SPHK1 in K562 cells. A. MTT assay was used to analyze the proliferation effect of K562 cells transfected with mimics NC, miR-659-3p mimics, miR-659-3p mimics and SPHK1. B. The proliferation ability was detected in K562 cells transfected with inhibitors NC, miR-659-3p inhibitors, miR-659-3p inhibitors and siSPHK1. C. Cell apoptosis was detected by AnnexinV-PI staining.

Then, we used the prediction algorithms of TargetScan to predict the target gene, and SPHK1 was found to be a target gene of miR-659-3p. The miR-659-3p regulated SPHK1 expression through binding to the 3'-untranslational region (3'-UTR). SPHK1 gene is located on human chromosome 17 (17q25.2) and distributed in the cytosol and the plasma membrane. Growth factors, cytokines, hormones and adhesion molecules can up-regulate SPHK1 and make it exhibit intrinsic catalytic activity [22]. A previous study has showed that SPHK1 high expression in tumors is closely associated with poor survival in glioblastoma, gastric cancer, breast cancer and cholangiocarcinoma [23]. In addition, studies have demonstrated that SPHK1 is an important enzyme encoded during neoplastic transformation [24, 25] and it plays

a critical role in motility and invasion of some cancer cells [15-17] including K562 cells by regulating the sphingolipid rheostat. Therefore, these findings provide a powerful rationale for targeting SPHK1 in CML [26-29].

In the present study, we identified that SPHK1 serves as a direct target gene of miR-659-3p in K562 cells. SPHK1 expression was upregulated in CML, and its expression level was negatively correlated with the expression level of miR-659-3p. To further confirm that miR-659-3p can directly target SPHK1, a knockdown plasmid of SPHK1 (siSPHK1) was used. Our results suggested that miR-659-3p inhibitors promoted the *in vitro* and decreased the cell apoptosis capacity of K562 cells whereas si-SPHK1 decreased this effect. These results altogether suggest that miR-659-3p was an upstream regulator of SPHK1. MiR-659-3p, by targeting SPHK1, inhibited the proliferation ability of K562 cells and promoted the apoptosis capacity of K562 cells, suggesting that miR-659-3p as a tumor suppressor played a key role in the motility of CML. Therefore, miR-659-3p may provide a potential therapeutic target for CML therapy in the future.

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Disclosure of conflict of interest

None.

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References

[1] Pane F, Mostarda I, Selleri C, Salzano R, Raiola AM, Luciano L, Saglio G, Rotoli B and Salvatore F. BCR/ABL mRNA and the P210(BCR/ABL) protein are downmodulated by interferon-alpha in chronic myeloid leukemia patients. Blood 1999; 94: 2200-2207.

[2] Patnaik MM, Parikh SA, Hanson CA and Tefferi A. Chronic myelomonocytic leukaemia: a concise clinical and pathophysiological review. Br J Haematol 2014; 165: 273-286.

[3] Todoric-Zivanovic B, Marisavljevic D, Surace C, Cemerikic V, Markovic O, Krtolica K, Tatomirovic Z, Cikota B, Magic Z and Rocchi M. A Ph-negative chronic myeloid leukemia with a complex BCR/ABL rearrangement and a t (6; 9) (p21; q34.1). Cancer Genet Cytogenet 2006; 166: 180-185.

[4] Jiang X, Zhao Y, Smith C, Gasparetto M, Turhan A, Eaves A and Eaves C. Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. Leukemia 2007; 21: 926-935.

[5] Melo JV, Gordon DE, Cross NC and Goldman JM. The ABL-BCR fusion gene is expressed in chronic myeloid leukemia. Blood 1993; 81: 158-165.

[6] Savage DG and Antman KH. Imatinib mesylate-a new oral targeted therapy. N Engl J Med 2002; 346: 683-693.

[7] Kantarjian H, Schiffer C, Jones D and Cortes J. Monitoring the response and course of chronic myeloid leukemia in the modern era of BCR-ABL tyrosine kinase inhibitors: practical advice on the use and interpretation of monitoring methods. Blood 2008; 111: 1774-1780.

[8] Hehlmann R. How I treat CML blast crisis. Blood 2012; 120: 737-747.

[9] Shah MY and Calin GA. MicroRNAs as therapeutic targets in human cancers. Wiley Interdiscip Rev RNA 2014; 5: 537-548.

[10] Zaman MS, Maher DM, Khan S, Jaggi M and Chauhan SC. Current status and implications of microRNAs in ovarian cancer diagnosis and therapy. J Ovarian Res 2012; 5: 44.

[11] Baer C, Claus R and Plass C. Genome-wide epigenetic regulation of miRNAs in cancer. Cancer Res 2013; 73: 473-477.

[12] Farazi TA, Hoell JI, Morozov P and Tuschl T. MicroRNAs in human cancer. Adv Exp Med Biol 2013; 774: 1-20.

[13] Mulrane L, Klinger R, McGee SF, Gallagher WM and O'Connor DP. microRNAs: a new class of breast cancer biomarkers. Expert Rev Mol Diagn 2014; 14: 347-363.

[14] Rosa R, Marciano R, Malapelle U, Formisano L, Nappi L, D'Amato C, D'Amato V, Damiano V, Marfe G, Del Vecchio S, Zannetti A, Greco A, De Stefano A, Carlomagno C, Veneziani BM, Troncone G, De Placido S and Bianco R. Sphingosine kinase 1 overexpression contributes to cetuximab resistance in human colorectal cancer models. Clin Cancer Res 2013; 19: 138-147.

[15] Pan J, Tao YF, Zhou Z, Cao BR, Wu SY, Zhang YL, Hu SY, Zhao WL, Wang J, Lou GL, Li Z, Feng X and Ni J. An novel role of sphingosine kinase-1 (SPHK1) in the invasion and metastasis of esophageal carcinoma. J Transl Med 2011; 9: 157.

[16] Sarkar S, Maceyka M, Hait NC, Paugh SW, Sankala H, Milstien S and Spiegel S. Sphingosine kinase 1 is required for migration, proliferation and survival of MCF-7 human breast cancer cells. FEBS Lett 2005; 579: 5313-5317.

[17] Bao M, Chen Z, Xu Y, Zhao Y, Zha R, Huang S, Liu L, Chen T, Li J, Tu H and He X. Sphingosine kinase 1 promotes tumour cell migration and invasion via the S1P/EDG1 axis in hepatocellular carcinoma. Liver Int 2012; 32: 331-338.

[18] Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nat Rev Genet 2009; 10: 704-714.

[19] Tanemura A, Terando AM, Sim MS, van Hoesel AQ, de Maat MF, Morton DL and Hoon DS. CpG island methylator phenotype predicts progression of malignant melanoma. Clin Cancer Res 2009; 15: 1801-1807.

[20] Sand M, Skrygan M, Georgas D, Sand D, Hahn SA, Gambichler T, Altmeyer P and Bechara FG. Microarray analysis of microRNA expression in cutaneous squamous cell carcinoma. J Dermatol Sci 2012; 68: 119-126.

[21] Xu N, Zhang L, Meisgen F, Harada M, Heilborn J, Homey B, Grander D, Stahle M, Sonkoly E and Pivarcsi A. MicroRNA-125b down-regulates matrix metallopeptidase 13 and inhibits cutaneous squamous cell carcinoma cell proliferation, migration, and invasion. J Biol Chem 2012; 287: 29899-29908.

[22] Pitson SM, Moretti PA, Zebol JR, Xia P, Gamble JR, Vadas MA, D'Andrea RJ and Wattenberg BW. Expression of a catalytically inactive sphingosine kinase mutant blocks agonist-induced sphingosine kinase activation. A dominant-negative sphingosine kinase. J Biol Chem 2000; 275: 33945-33950.

[23] Pyne NJ and Pyne S. Sphingosine 1-phosphate and cancer. Nat Rev Cancer 2010; 10: 489-503.

[24] Fuereder T, Hoeflmayer D, Jaeger-Lansky A, Rasin-Streden D, Strommer S, Fisker N, Hansen BJ, Crevenna R and Wacheck V. Sphingosine kinase 1 is a relevant molecular target in gastric cancer. Anticancer Drugs 2011; 22: 245-252.

[25] Madhunapantula SV, Hengst J, Gowda R, Fox TE, Yun JK and Robertson GP. Targeting sphingosine kinase-1 to inhibit melanoma. Pigment Cell Melanoma Res 2012; 25: 259-274.

[26] Baran Y, Salas A, Senkal CE, Gunduz U, Bielawski J, Obeid LM and Ogretmen B. Alterations of ceramide/sphingosine 1-phosphate rheostat involved in the regulation of resistance to imatinib-induced apoptosis in K562 human chronic myeloid leukemia cells. J Biol Chem 2007; 282: 10922-10934.

[27] Barnes DJ, Palaiologou D, Panousopoulou E, Schultheis B, Yong AS, Wong A, Pattacini L, Goldman JM and Melo JV. Bcr-Abl expression levels determine the rate of development of resistance to imatinib mesylate in chronic myeloid leukemia. Cancer Res 2005; 65: 8912-8919.

[28] le Coutre P, Tassi E, Varella-Garcia M, Barni R, Mologni L, Cabrita G, Marchesi E, Supino R and Gambacorti-Passerini C. Induction of resistance to the Abelson inhibitor STI571 in human leukemic cells through gene amplification. Blood 2000; 95: 1758-1766.

[29] Weisberg E and Griffin JD. Mechanism of resistance to the ABL tyrosine kinase inhibitor STI571 in BCR/ABL-transformed hematopoietic cell lines. Blood 2000; 95: 3498-3505.