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Overexpression of PKMYT1 indicates the poor prognosis and enhances proliferation and tumorigenesis in non-small cell lung cancer via activation of Notch signal pathway

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Abstract. – OBJECTIVE: The protein kinase, membrane-associated tyrosine/threonine 1 (PKMYT1) has been implicated as an important factor promoting the tumorigenesis of hepatocellular carcinoma and colorectal cancer. The current study was designed to explore the functional role of PKMYT1 in non-small cell lung cancer (NSCLC) cell behaviors and to investigate the possible molecular mechanisms.

PATIENTS AND METHODS: The expression levels of PKMYT1 in NSCLC tissues and cell lines were determined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The clinical and prognostic significance of PKMYT1 in 153 cases of NSCLC was determined. We also evaluated the effects of KMYT1 on NSCLC cell proliferation, migration, and invasion in vitro. Western blot was performed to assure whether PKMYT1 affected the Notch signal pathway and MET pathway.

RESULTS: We observed that PKMYT1 expression was significantly up-regulated in both NSCLC tissues and cell lines. Higher expression of PKMYT1 was associated with clinical stage and lymph nodes metastasis. Clinical survival assays demonstrated that patients with high PKMYT1 expression had a shorter overall survival time than those with low PKMYT1 expression. Moreover, the multivariate analysis confirmed that increased expression of PKMYT1 was an independent predictor of overall survival. Functionally, knockdown of PKMYT1 in the NSCLC cell lines A549 and H1299 suppressed NSCLC cells proliferation, invasion and migration, and promoted apoptosis. In addition, the down-regulation of PKMYT1 resulted in the inhibition of EMT in NSCLC cells. Further mechanistic studies revealed that when PKMYT1 was silenced, the expression levels of Notch1, p21, and Hes1 were respectively downregulated, suggesting that PKMYT1 could promote the activity of the Notch signal pathway.

CONCLUSIONS: PKMYT1 plays a significant role in NSCLC aggressiveness and clinical outcome, and may serve as a promising therapeutic target for this disease.

Key Words:
PKMYT1, NSCLC, Biomarker, Notch signal pathway, Metastasis, Apoptosis.

Introduction

Lung cancer is the leading cause of cancer-associated death and the most common type of malignancy worldwide, with about 1.4 million deaths reported annually[1,2]. In recent years, its incidence continued to rise gradually in the vast majority of countries, particularly in China, which has built a serious burden for human beings[3]. Non-small cell lung cancer (NSCLC), accounts for at least 80% of all cases of this malignancy[4]. The pathogenesis of tumors, including NSCLC, is very complex. Neoplastic cells and the tumor microenvironment were confirmed to be involved in lung carcinogenesis[5,6]. With recent advances in diagnostics and multimodal therapies, the five-year overall survival of patient diagnosed early stages has been improved[7,8]. Unfortunately, the high rate of recurrence or metastasis after surgery (40-65% at five years) hinders further improvements in the prognosis of NSCLC patients[9,10]. Thus, it is vital to clarify the molecular mechanism of NSCLC progression to develop effective treatments and prognostic markers.

The protein kinase, membrane-associated tyrosine/threonine 1 (PKMYT1), encoded by the PKMYT1 gene, is located at 16p13.3[11]. As a member of the serine/threonine protein kinase family, the main function of PKMYT1 is to preferentially phosphorylates and inactivates cyclin-dependent kinase 1 (CDK1) which was firstly identified in

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a landmark genetic screen for genes that are involved in the regulation of the cell cycle[12,13]. The phosphorylation of CDK1 results in the inactivation of CDK1, leading to a barricade of the transition from G2 to the cells phase[14]. A large number of evidence indicated that CDK1 plays important roles in the progression of various tumors[15,16]. Thus, PKMYT1 may influence the progress of the tumor by regulating the cell cycle via the phosphorylation of CDK1. It was reported[17] that PKMYT1 was highly expressed in hepatocellular carcinoma and acted as a tumor promoter by activating beta-catenin/TCF signaling. However, the studies of PKMYT1 on tumors were rare. Whether PKMYT1 was involved in the modulation of NSCLC progression remains largely unclear.

In this research, we firstly reported that PKMYT1 expression was significantly up-regulated in both NSCLC tissues and cell lines. Then, we explored its clinical significance in NSCLCL patients and potential function in the proliferation, migration, and invasion of NSCLC cells. Our findings suggested the potential use of PKMYT1 for the prediction of prognosis and the treatment of NSCLC.

Patients and Methods

Patients and Tissue Samples

NSCLC tissues with the matched normal adjacent specimens were obtained from 153 NSCLC patients at The Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University, from June 2010 to March 2013. The patients did not receive radiation therapy or chemotherapy prior to surgery. For the purpose of using these clinical materials for scientific research, we obtained both patients informed consent and approval from the Institutional Research Ethics Committee of The Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University. The clinical information was shown in Table I.

Cell Lines and Cell Transfection

Human epithelial cell type, BEAS-2B, and five NSCLC cell lines: A549, HCC827, H1299, SPC-A1, and H1975, were all cultured using Roswell Park Memorial Institute-1640 (RPMI-1640) medium with 10% fetal bovine serum (FBS) and 1% antibiotics, obtained from YiHui Biological Co., Ltd. (Minhang, Shanghai, China). Small interfering RNAs (siRNAs) against PKMYT1 (siRNA#1, siRNA#2) and negative control siRNAs (NC-siRNA), which were obtained from BioTend Co., Ltd. (Huangpu, Shanghai, China), were transfected into A549, as well as H1299 cells using Lipofectamine 3000 reagent with standard protocols.

Real-Time PCR Assays

We extracted total RNA from A549 and H1299 cells treated with corresponding siRNAs using TRIzol reagent (KINDU Biotech, Minhang,

Table I. Correlation between PKMYT1 expression and clinicopathological features of NSCLC.

Variable	Number	PKMYT1 expression		*p*-value
		Low	High	
Age (years)				0.462
< 60	79	38	41	
≥ 60	74	40	34	
Gender				0.144
Male	93	43	50	
female	60	35	25	
Tumor size (cm)				0.089
< 3	98	55	43	
≥ 3	55	23	32	
Histologic type				0.800
SCC	73	38	35	
AD	80	40	40	
Clinical stage				0.017
I-II	100	58	42	
III	53	20	33	
Lymph nodes metastasis				0.026
No	106	60	46	
Yes	46	17	29	

Table II. Primers sequence for qRT-PCR assay.

Name	Sequence (5′-3′)	
PKMYT1	Forward	CATGGCTCCTACGGAGAGGT
	Reverse	ACATGGAACGCTTTACCGCAT
GAPDH	Forward	TGTAGTTGAGGTCAATGAAGGG
	Reverse	ACATCGCTCAGACACCATG

Shanghai, China). Then, the synthesis of cDNA was conducted using Roche Transcriptor First Strand cDNA Synthesis kit (FeiHeng, Minhang, Shanghai, China). Afterward, qRT-PCR assays were carried out using the SYBR Green qPCR assay kit (CWBIO, Changping, Beijing, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was deemed as internal control. The expression levels of PKMYT1 were examined as relative fold changes using the $2^{-\Delta\Delta Ct}$ method. The primers used in this study were shown in Table II.

Cell Counting Kit-8 (CCK-8) Assays

First, we transfected PKMYT1 siRNAs or NC siRNAs into A549 and H1299 cells. Then, the transfected cells were collected and transferred to 96-well plates (2×10^3 cells/well). Thereafter, the cell proliferation was examined by Dojindo CCK-8 reagent (10 µl/well; BoGoo, Putuo, Shanghai, China) at 1, 2, 3, and 4 days of cultivation. Finally, the OD 450 nm was recorded using a PT-3502 microplate reader system (POTENOV, Tongzhou, Beijing, China).

Cell Colony Formation Assays

The colony formation capacity of PKMYT1 siRNAs or NC siRNAs-transfected A549 and H1299 cells were evaluated by the cell colony formation assay. In total, about 500 cells (per well) suspended in 1.2 ml complete medium were plated in 6-well plates. These plates were then incubated for about 14 days until the colonies were observed. Then, we stained the colonies with crystal violet dye (0.1%; ABSIN, Pudong, Shanghai, China) and took photographs using an FRD-4C microscope (COSSIM Scientific Instrument, Chaoyang, Beijing, China).

Flow Cytometry Analysis

The cell cycle distribution and apoptosis were determined by flow cytometry. In brief, the A549 and H1299 cells were first placed in 6-well plates for 24 h, and then the PKMYT1 siRNAs or NC siRNAs were transfected into these cells. Thereafter, the transfected cells were harvested by centrifugation and resuspended in binding buffer for cell apoptosis or cell cycle examination. Subsequently, 10 µl annexin V-FITC/PI reagent (for cell apoptosis detection) or 5 µl PI buffer (with 2 µl RNase A) was separately added into the cells and the cells were kept in the dark for 20 min. Next, the cells were analyzed by a BD LSRFortessa X-20 flow cytometer (New Discovery, Pudong, Shanghai, China). The annexin V-FITC, as well as PI were all purchased from Beyotime Biotechnology Co., Ltd. (Haimen, Jiangsu, China).

Western Blot Assays

We extracted total proteins from the PKMYT1 siRNAs or NC siRNAs-transfected A549 and H1299 cells using the BugBuster Protein Extraction Reagent (Sigma-Aldrich, Pudong, Shanghai, China). Subsequently, the cell lysates were subjected to 8-12% of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (PVDF; Millipore, Billerica, MA, USA). After being blocked with 5% bovine serum albumin (BSA), the membranes were separately incubated with primary antibodies against vimentin, β-catenin, Notch1, p2, and Hes1, followed by matched secondary antibodies incubation. The protein bands were then examined by a BeyoECL Plus assay kit (Beyotime, Haimen, Jiangsu, China). We obtained all the antibodies used in this study from Univ Biotechnology Co., Ltd. (Nanjing, Jiangsu, China).

Wound Healing Assays

3×10^5 (per well) A549 or H1299 cells were placed into 6-well plates and grown to appropriate 70% of cell confluency. Subsequently, PKMYT1 siRNAs or NC siRNAs were separately transfected into A549 or H1299 cells. 32-48 h post-transfection, a 'wound' was generated by the use of a 200 µl plastic pipette tip scratching the cell monolayer. Finally, a microscope (FRD-4C; COSSIM Scientific Instrument, Chaoyang, Beijing, China) was utilized to record the average distance of migration cells.

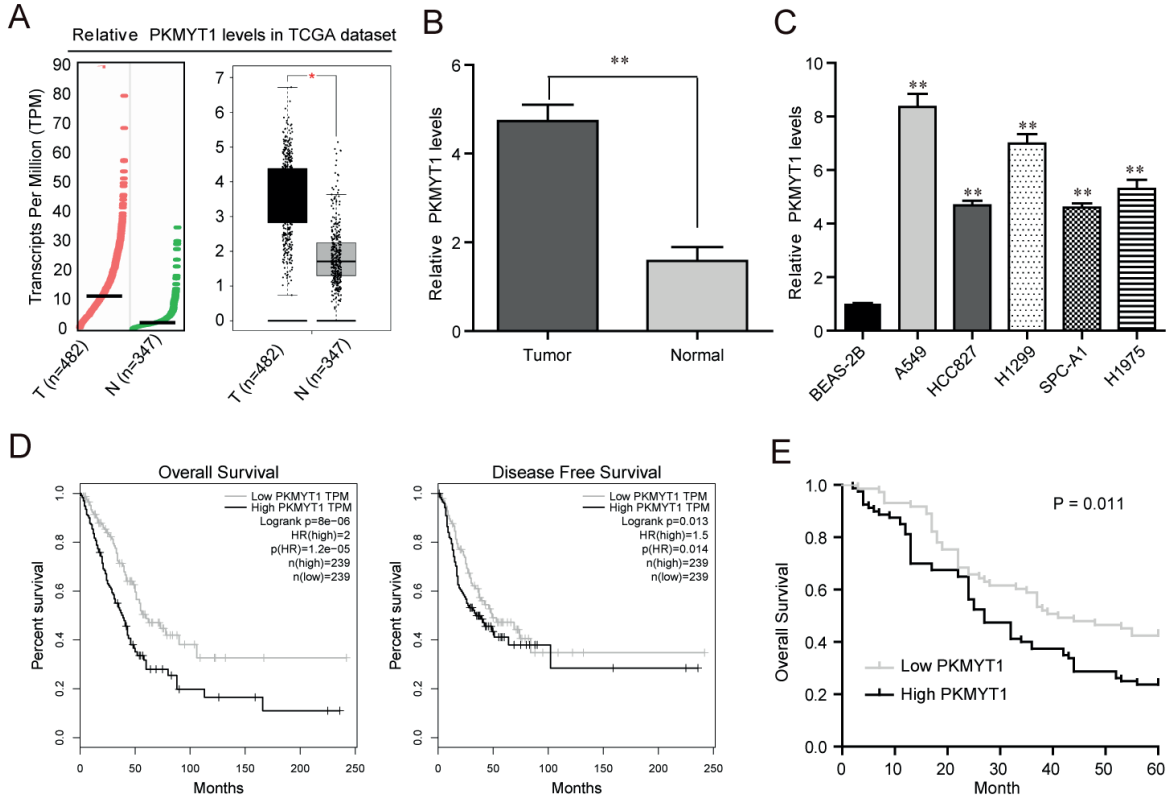

Figure 1. PKMYT1 was highly expressed in NSCLC tissues and cells. **A,** PKMYT1 expression in lung cancer tissues (n=482) compared with noncancerous tissues (n=347) analyzed using GEPIA tool. **B,** Expression levels of PKMYT1 in 153 pairs of NSCLC tissues and their matched normal tissues were determined by RT-PCR. **C,** Expression level of PKMYT1 in five NSCLC cell lines and normal BEAS-2B cells. Assays were performed in triplicate. **D,** Association between PKMYT1 expression and overall survival and disease-free survival analyzed using GEPIA tool. **E,** Overall survival time was observed in patients with a low PKMYT1 expression compared to a higher PKMYT1 expression patients as shown by the Kaplan-Meier survival curve. * $p < 0.05$, ** $p < 0.01$.

Transwell Invasion Assays

The invasion of A549 and H1299 cells after treatment were conducted using a Corning transwell plate (pore size: 8 μm; pre-coated with Matrigel). Briefly, the cells were collected and resuspended in a serum-free medium, followed by being transferred into the upper chamber. As a chemoattractant, the 15% FBS was added into the lower chamber. 48 h later, the cells on the lower side of the chamber membrane were stained with 0.1% crystal violet and counted using a microscope.

Statistical Analysis

Statistical analysis was done with SPSS/Win 11.0 software (SPSS Inc., Chicago, IL, USA). The statistical significance between groups was determined using a two-tailed Student's *t*-test. One-way ANOVA and Tukey post-hoc test was performed to analyze the difference between three or above groups. The relation between PKMYT1 expression and various clinicopathological parameters were analyzed by the Chi-square test. Kaplan-Meier analysis and the log-rank test were carried out to identify survival differences in NSCLC patients. The HR and significance between variables and overall survival were carried out using the Cox proportional hazards model. A *p*-value< 0.05 was considered as statistically significant.

Results

PKMYT1 Expression is Increased in NSCLC Tissues and Cell Lines

We downloaded the microarray data from the Cancer Genome Atlas (TCGA) datasets and screened abnormally expressed mRNAs. As shown in Figure 1A, we found that PKMYT1 expression

was significantly up-regulated in NSCLC tissues. Then, a total of 153 paired clinical NSCLC samples and adjacent normal lung samples were analyzed for PKMYT1 expression by using qRT-PCR. Figure 1B revealed that the expression of PKMYT1 was distinctly increased in NSCLC samples compared to matched normal lung samples ($p < 0.01$). Moreover, PKMYT1 in A549, HCC827, H1299, SPC-A1, and H1975 cells was detected using Real Time PCR. As expected, we observed that PKMYT1 was significantly increased in five NSCLC cells compared with BEAS-2B cells (Figure 1C). Thus, our findings revealed that PKMYT1 was highly expressed in NSCLC and may act as an oncogene.

High PKMYT1 Expression Predicts Poor Prognosis in Patients with NSCLC

We explored the clinical significance of PKMYT1 in NSCLC patients. NSCLC samples were classified into low PKMYT1 expression group (n = 78) and high PKMYT1 expression group (n = 75) based on the median PKMYT1 expression level of all NSCLC samples. As shown in Table I, we observed that high PKMYT1 expression was associated with positive lymph nodes metastasis and ($p = 0.026$) and advanced clinical stage ($p = 0.017$), but high PKMYT1 levels were not distinctly associated with other clinical parameters such as age and gender ($p > 0.05$). Furthermore, we used bioinformatics by internet which downloaded clinical data of survival from TCGA datasets to further analyze the prognostic value of PKMYT1 in NSCLC patients, finding that higher expression of PKMYT1 was distinctly correlated with shorter overall survival and disease-free survival (Figure 1D). To further explore whether PKMYT1 expression could influence the clinical outcomes of NSCLC patients, the clinical follow-up was available for all NSCLC patients. The result of Kaplan-Meier assays suggested that NSCLC patients with higher PKMYT1 expression level had a shorter overall survival than those with high PKMYT1 expression level ($p = 0.011$). Notably, univariate analysis of the overall survival suggested that the relative levels of PKMYT1 expression, clinical stage, and lymph nodes metastasis were prognostic indicators (Table III). Then, further multivariate analysis was used to analyze these variables with a value of $p < 0.05$. As shown in Table III, the results confirmed that high PKMYT1 expression (HR=3.015, 95% CI: 1.328-4.667, $p = 0.006$) was a significant independent prognostic biomarker of poorer survival in NSCLC.

Effects of PKMYT1 on Cellular Growth and Apoptosis of Lung Cancer Cells

According to the high expression of PKMYT1 in lung cancer tissues and cell lines, we next performed loss-function assays using A549 and H1299 cells to investigate the biological functions of PKMYT1 on lung cancer cells. Firstly, the siRNAs targeting PKMYT1 (siRNA#1 and siRNA#2) were synthesized and transfected into A549 and H1299 cells. Subsequently, the qRT-PCR analysis was conducted and the data suggested that the knockdown efficiency of PKMYT1 siRNAs were high (Figure 2A). Results from the CCK-8 assays revealed that PKMYT1 siRNAs transfection significantly decreased the cellular growth curves of A549 and H1299 cells, compared to the negative controls (Figure 2B). In addition, data from cell colony forming assays demonstrated that repressing PKMYT1 expression levels remarkably reduced the cell colony number of both A549 and H1299 cells (Figure 2C). Furthermore, according to the results of cell cycle analysis, the transfection of PKMYT1 siRNAs markedly increased the S phase cell number in A549 and H1299 cells, indicating that silence of PKMYT1 induced S phase arrest of lung cancer cells (Figure 2D). Besides, we also determined the impact of PKMYT1 knock-

Table III. Summary of univariate and multivariate Cox regression analyses of overall survival.

	Univariate analysis			Multivariate analysis		
Parameter	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Age (< 60 *vs.*≥60)	1.653	0.572-2.331	0.154	-	-	-
Gender (Male vs. Female)	1.348	0.673-2.219	0.228	-	-	-
Tumor size (< 3 *vs.*≥3)	1.548	0.732-2.341	0.138	-	-	-
Histologic type (SCC *vs.* AD)	1.741	0.669-2.562	0.126	-	-	-
Clinical stage (I-II *vs* III)	3.342	1.436-4.781	0.008	2.894	1.211-4.137	0.025
Lymph nodes metastasis (No *vs.* Yes)	3.451	1.348-4.665	0.007	2.946	1.157-4.325	0.011
PKMYT1 expression (Low *vs.* High)	3.377	1.528-5.214	0.003	3.015	1.328-4.667	0.006

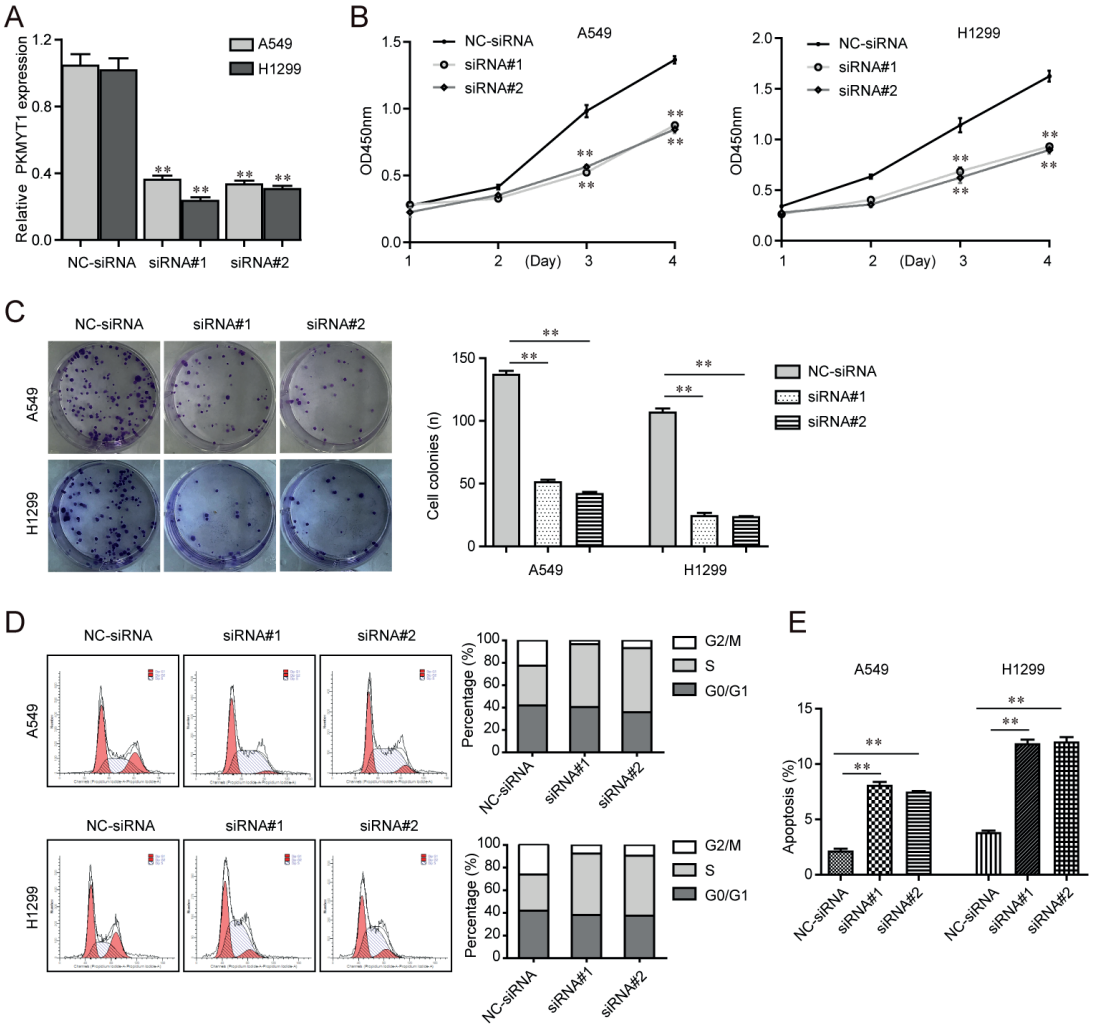

Figure 2. Knockdown of PKMYT1 inhibited the cell proliferation and induced the apoptosis of A549 and H1299 cells. **A,** QRT-PCR assays detected the relative PKMYT1 levels in A549 and H1299 cells after transfecting with siRNAs targeting PKMYT1 (siRNA#1 and siRNA#2) and negative control siRNAs (NC-siRNA). **B,** Growth curves of A549 and H1299 cells at 48 h, 72 h, and 96 h were examined by CCK-8 assays. **C,** Colony formation assays evaluated the colony formation capabilities of A549 and H1299 cells after siRNAs transfection (Magnification: 10×). **D,** Cell cycle analysis was conducted by flow cytometry. **E,** Flow cytometry determined the cell apoptosis. * $p < 0.05$, ** $p < 0.01$.

down on cellular apoptosis using flow cytometry. Results showed that the apoptotic rates of A549 and H1299 cells were significantly elevated when the cells were transfected with PKMYT1 siRNAs (Figure 2E). To sum up, these data indicated that PKMYT1 acted as critical roles in the regulation of lung cancer cell proliferation and apoptosis.

PKMYT1 Modulated the Migration and Invasion of Lung Cancer Cells

We ascertained if PKMYT1 deficiency affected the metastatic potentials of lung cancer cells. Hence, we conducted wound healing and transwell invasion assays. As the data from wound healing assays presented in Figures 3A and B, the wound closure was remarkably inhibited in the A549 and H1299 cell monolayers when the cells were transfected with PKMYT1 siRNAs, suggesting that silence of PKMYT1 suppressed the migratory capacity of A549 and H1299 cells. Additionally, the results of transwell invasion assays displayed that down-regulation of PKMYT1 markedly reduced the invasive cell number of A549 and H1299 cells (Figure 3C). Since migration and invasion were important features of the epithelial-mesenchymal transition (EMT), we wondered whether PKMYT1 depletion was capable to affect the EMT relevance molecules. To this

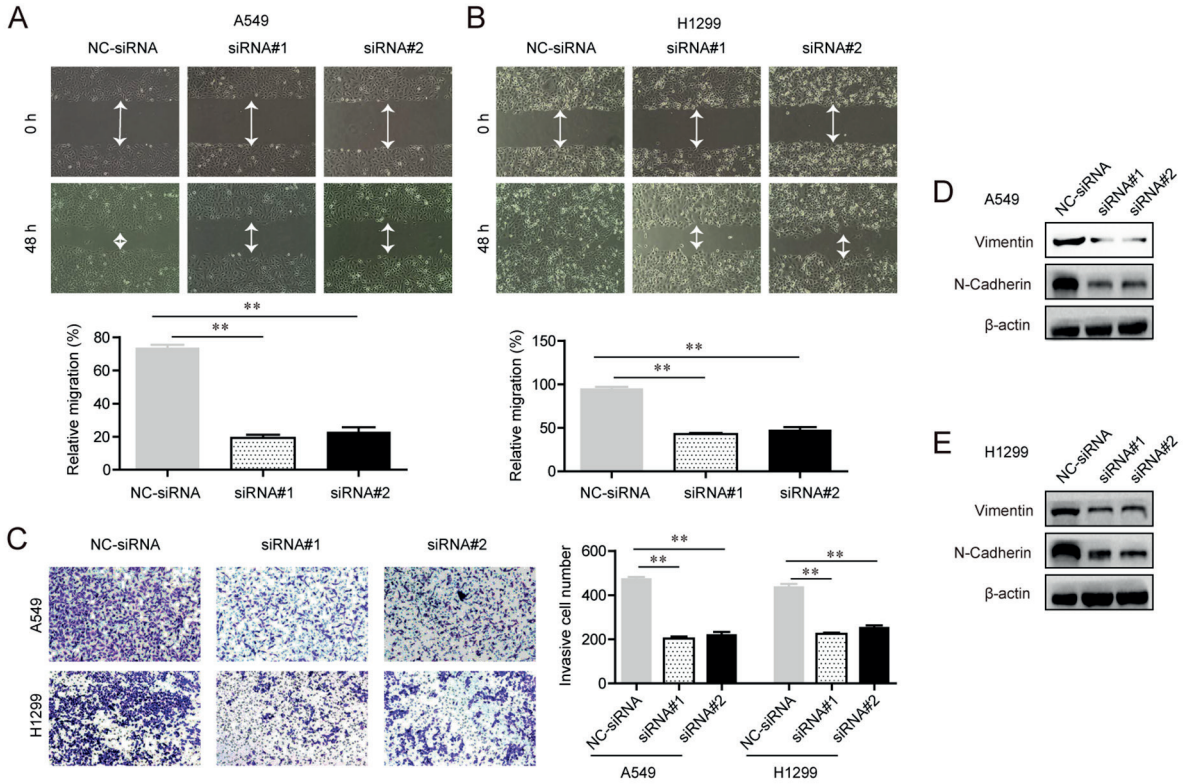

Figure 3. PKMYT1 silence inhibited the invasion and migration of A549 and H1299 cells. **A** and **B,** Migratory capacity of A549 and H1299 cells was decreased after they were transfected with PKMYT1 siRNAs (Magnification: 10×). **C,** After knockdown of PKMYT1 siRNAs, the invasion ability of A549 and H1299 cells was decreased (Magnification: 40×). **D** and **E**, Protein expression of A549 and H1299 cells was determined by Western blot analysis. * $p < 0.05$, ** $p < 0.01$.

purpose, Western blot analysis was carried out and the results demonstrated that the expression of N-cadherin and vimentin was significantly decreased in A549 and H1299 cells when they were transfected with PKMYT1 siRNAs (Figures 3D and E). Therefore, these data revealed that PKMYT1 was able to affect the metastatic potentials of lung cancer cells via EMT.

Knockdown of PKMYT1 Suppressed the Activity of Notch Signaling in Lung Cancer Cells

We attempted to uncover the detail molecular mechanisms by which PKMYT1 had a critical impact on the cellular growth, apoptosis, and mobility of lung cancer cells. Considering that the alterations of Notch signaling components were

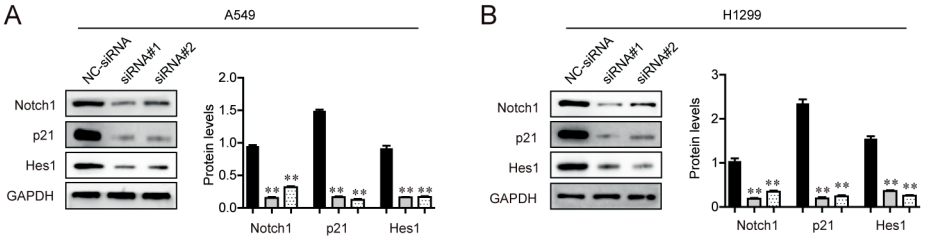

Figure 4. The activity of Notch signaling pathway in A549 and H1299 cells was depressed by silence of PKMYT1. **A,** Protein levels of Notch1, p21, and Hes1 in A549 cells were detected by Western blot analysis. The optical density of the proteins was analyzed by Image J (NIH, Bethesda, MD, USA). **B,** Western blot assays determined corresponding protein levels in H1299 cells. * $p < 0.05$, ** $p < 0.01$.

relevance with tumor development and progression in various cancer types, we next conducted Western blot analysis to evaluate the expression levels of some key components such as Notch1, p21, and Hes1, involved in the Notch signaling. As the data presented in Figure 4A, the repressed expression of PKMYT1 in A549 cells resulted in notably decreased protein levels of Notch1, p21, and Hes1. Similarly, as validated by Western blot assays using H1299 cells, transfection of PKMYT1 siRNAs markedly reduced the expression of Notch1, p21, and Hes1 (Figure 4B). Therefore, our data suggested that the activity of Notch signaling in lung cancer cells was inhibited by PKMYT1 deficiency.

Discussion

Up to date, the prognosis of NSCLC remains poor and the five-year survival rate is less than 15%. To improve the management of NSCLC, the identification of novel biomarkers and critical target molecules of NSCLC metastasis are two critical steps for the prediction of prognosis and targeted therapy of NSCLC patients[18,19]. Despite a long list of potential biomarker candidates which have been frequently reported in various references, not one sensitive biomarker has been widely approved for routine clinical application in managing patients with NSCLC[20,21]. Thus, more exploration of new biomarkers is necessary in clinical and basic studies.

In this research, we focused on a new protein PKMYT1 which can modulate the expression of CDK1. We found that PKMYT1 expression was significantly up-regulated in both NSCLC tissues and cell lines. Clinical investigation revealed that higher expression of PKMYT1 was associated with advanced clinical stage, lymph nodes metastasis and shorter overall survival. In addition, the clinical data from TCGA datasets also confirmed our results that PKMYT1 was associated with the clinical prognosis of NSCLC patients. Notably, univariate and multivariate analysis further confirmed that PKMYT1 expression was an independent poor prognostic factor for the five-year overall survival, suggesting that PKMYT1 have the potential to be a promising biomarker for NSCLC patients. However, due to the small sizes of samples, further research with more patients was needed to confirm our findings.

Studies on the effects of PKMYT1 in tumors were limited. In colorectal cancer, PKMYT1 was found to be up-regulated and predicted a shorter overall survival of patients[22]. Besides, Liu et al[17] reported that in hepatocellular carcinoma, increased expression of PKMYT1 promoted the growth, metastasis and EMT pathway by activating beta-catenin/TCF signaling. These findings suggested that PKMYT1 had important biological functions in carcinomas as being oncogenic. However, the association between PKMYT1 and NSCLC progression remains largely unclear. In this work, A549 and H1299 cells were transfected with si-PKMYT1 to study the function of PKMYT1 in NSCLC cells. We found that suppression of PKMYT1 suppressed the proliferation and led to a significant increase in the number of colonies formed by A549 and H1299. Using flow cytometry, we found that down-regulation of PKMYT1 induced cell cycle arrest at the G0/1 transition. What's more, the knockdown of PKMYT1 may have a facilitation effect on apoptosis of A549 and H1299 cells. These findings demonstrated the oncogenic roles of PKMYT1 in NSCLC growth. Then, we also confirmed that the inhibition of PKMYT1 suppressed the migration and invasion of A549 and H1299 cells. Cancer progression and metastasis are distinctly associated with the mortality of patients with NSCLC. The EMT is an initial step toward tumor metastasis and may be an identified phenotype of tumor metastasis in malignancies[23,24]. In this work, we observed that knockdown of PKMYT1 resulted in decreased E-cadherin expression level and Vimentin, suggesting that PKMYT1 displayed its oncogenic roles in the progress of metastasis by promoting the EMT progress. However, the underlying mechanism linking PKMYT1 and EMT should be further investigated.

The Notch is a well-conserved signaling pathway all through evolution that has been confirmed to be positively involved in embryo development through the regulation of cell proliferation, differentiation, angiogenesis and stemness[25,26]. Overexpression of Notch elements such as receptors, ligands, and downstream targets genes, is associated with advanced progress, metastatic potential, recurrence and clinical prognosis in almost all types of tumors[27-29]. Many tumor-related genes are found to target the key components of Notch signaling pathway and, thus, modulate tumor cells progression[30,31]. To explore the potential mechanism by which PKMYT1 promoted the proliferation, metastasis, and apoptosis, our attention focused on the association between PKMYT1 and Notch signaling pathway. We found that inhibi-

tion of PKMYT1 resulted in the down-regulation of mRNA expression of Notch signal-related proteins including Notch-1, Jag-1, and Hes-1, which suggested that PKMYT1 exhibited oncogenic roles in NSCLC cells via modulating the Notch signaling pathway. However, functional mechanisms are not deeply researched in this article.

Conclusions

We firstly indicated that PKMYT1 is significantly overexpressed and associated with poor prognosis of NSCLC patients. Moreover, the knockdown of PKMYT1 suppressed the proliferation, migration, and invasion via modulating the Notch signaling pathway. This research revealed the vital significance of PKMYT1 in NSCLC development, which provided a novel biomarker and potential therapeutic target for NSCLC.

Conflict of interest

The authors declare no conflicts of interest.

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