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ORIGINAL RESEARCH

PRMT7 contributes to the metastasis phenotype in human non-small-cell lung cancer cells possibly through the interaction with *HSPA5* and *EEF2*

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Background: Non-small-cell lung cancer (NSCLC) constitutes the leading cause of cancer death in humans. Previous studies revealed the essential role of the protein arginine methyltransferase 7 (*PRMT7*) in promoting metastasis in breast cancer. However, its function and potential mechanism in NSCLC remain unclear.

Materials and methods: The gene expression of *PRMT7* between lung cancer tissues and normal tissues was studied with online database (http://medicalgenome.kribb.re.kr/GENT/). NSCLC cell lines with specific gene overexpression were constructed with lentivirus transduction. Matrigel invasion and colony formation assays were performed to evaluate the invasion and colony formation abilities. Co-immunoprecipitation coupled with mass spectrometry analysis was performed to explore the potential interaction proteins of *PRMT7*. Bioinformatic analysis was performed with Gene Ontology and Kyoto Encyclopedia of Genes and Genomes databases.

Results: Online analysis of gene expression patterns revealed the relatively high expression of *PRMT7* in lung cancer tissues. *PRMT7* overexpression was able to promote the invasion and colony formation of A549 and SPC-A1 cells. A total of 19 in-common proteins shared by both NSCLC cell lines were identified to be interacting with *PRMT7* and found to participate in a wide variety of pathways and protein–protein interactions according to bioinformatic analysis. Among them, *HSPA5* and *EEF2* were further investigated for their essential roles in *PRMT7*-promoted NSCLC cell invasion.

Conclusion: Our results suggested *PRMT7* overexpression was able to promote metastasis in NSCLC possibly through the interaction with *HSPA5* and *EEF2*, which provides the potential mechanism of oncogenesis in lung cancer.

Keywords: human non-small-cell lung cancer, arginine methylation, *PRMT7*, protein–protein interaction, *HSPA5*, *EEF2*

Introduction

Lung cancer, characterized by a poor prognosis, is among the most deadly cancers all over the world,[1] which can be divided into two main subtypes, small-cell lung carcinoma and non-small-cell lung carcinoma (NSCLC).[2] Of these, NSCLC accounts for the majority (>80%) of lung cancer cases. More importantly, NSCLC is often detected in the late stage of metastasis, which makes effective treatment impossible.[3] In a previous report, it was suggested that metastasis in lung cancer is a multifaceted process, which still remains unclear in respect to the molecular basis of mechanism.[4] Together, these represent great challenges in the study of NSCLC as well as in its clinical management.[5]

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Arginine methylation, a common posttranslational modification,[6] includes three types of methylarginine products in mammalian cells, for example, ω-N^G-monomethylarginine, ω-N^G,N^G-asymmetric dimethylarginine, and ω-N^G,N'^G-symmetric dimethylarginine.[7] The formation of methylarginine is catalyzed by the family of protein arginine methyltransferases (PRMTs), which consist of nine different members and can be divided into three different types (types I, II, and III).[7,8] Accumulating studies have indicated the deregulation of PRMTs is implicated in the pathogenesis of cancers.[7,9–11]

PRMT7 is a unique member characterized by both type II and type III enzyme activities.[7] *PRMT7* has been shown to be extensively involved in a wide variety of biological processes,[12] such as DNA transcription, RNA splicing, DNA damage repair, metastasis, and pluripotency.[13–15] Moreover, *PRMT7* is required for the snRNP assembly, which is a major component of the spliceosome.[16] Recently, *PRMT7* was recognized as a metastasis promoter gene in breast cancer, which could be attributed to the induction of epithelial-to-mesenchymal transition by *PRMT7*.[17,18] Furthermore, Jelinic et al demonstrated *PRMT7* was able to interact with the proposed oncogene CCCTC binding factor-like (CTCFL),[19] which increased its methyltransferase activity.[20] *PRMT7* also showed promise in the anticancer therapies, which could be supported by the fact that *PRMT7* downregulation sensitized Hela cells to the inhibitor of DNA topoisomerase I, camptothecin.[21]

However, little is known about the function of *PRMT7* in NSCLC, despite the knowledge on its participation in numerous essential processes. To investigate its potential role, we constructed the *PRMT7*-overexpressed NSCLC cell models and evaluated its effects on cancer metastasis. We also explored the interacting proteins of *PRMT7*, among which *HSPA5* and *EEF2* were highlighted owing to the established contributions to the invasion of NSCLC cells. Our findings laid the basis for further study of the molecular basis of mechanism, which could help in the development of targeted chemotherapy against NSCLC.

Materials and methods
Cell lines
Human lung adenocarcinoma A549 and SPC-A1 cells were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). HEK293T cells were purchased from ATCC (No. CRL-11268; American Type Culture Collection, Manassas, VA, USA). Cells were grown in DMEM medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Thermo Fisher Scientific), 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin (Mediatech Inc., Manassas, VA, USA) in a humidified atmosphere of 5% CO_2 at 37°C.

PRMT7 expression in lung cancer tissues
The analysis of gene expression of *PRMT7* in both normal and cancer lung tissues was performed with the online database Gene Expression across Normal and Tumor tissue (http://medicalgenome.kribb.re.kr/GENT/).[22]

Vector construction
RPMT7 overexpression vector was constructed by cloning the cDNAs of human RPMT7 into a PSE2130 vector. The constructs were confirmed by sequencing.

Gene overexpression and knockdown
pSE2130 retroviral vectors were used to study gene overexpression of RPMT7. *EEF2* and *HSPA5* were knocked down with a pLVT1568-based lentiviral vector. Lentiviruses were generated in HEK293T cells as previously described.[23] A549 and SPC-A1 cells were infected with virus-containing supernatants. Alterations in the expression of RPMT7, *EEF2*, and *HSPA5* were confirmed by Western blotting or quantitative reverse transcriptase-polymerase chain reaction.

RNA isolation and quantitative reverse transcriptase-polymerase chain reaction
Total RNA was extracted from the cultured cells with TRIzol reagent according to the standard manual. RNA was reverse transcribed using Revert Aid First Strand cDNA Synthesis Kit before quantification with spectrophotometry. Maxima SYBR Green/ROX qPCR Master Mix was used for real-time polymerase chain reaction experiment. GAPDH was used as an internal control.

Cell invasion assay
Cells' invasion ability was evaluated as described previously.[24] Briefly, cells (1×10^5) cultured in 100 μL FBS-free medium were seeded into transwell chambers which were precoated with Matrigel. Medium containing 20% FBS was added to the lower chamber. After culturing for 72 h at 37°C, non-invading cells were removed with cotton swabs. Invading cells were fixed with 4% para-formaldehyde and stained with crystal violet solution. Data were quantified by ImageJ.

Colony formation assay
Colony formation assays were performed as described before.[25] Briefly, 2 mL of 250 cells per well were seeded into

a six-well plate. After culturing in the cell incubator (37°C, 5% CO_2) for 10 days, the colonies per well were fixed with ethanol, stained with crystal violet solution, and observed with a microscope. Data were quantified by ImageJ.

Immunoprecipitation and liquid chromatography with tandem mass spectrometry protein identification

Immunoprecipitation was carried out as previously reported.[26] Cells overexpressing RPMT7 were harvested and lysed with immunoprecipitation (IP) lysis buffer (Biyuntian, Shanghai, China). After centrifugation at 16,000× *g* at 4°C for 15 minutes, the supernatants were collected and the protein concentration was quantified with bicinchoninic acid reagent. Then, the samples were pre-cleared with protein A/G magnetic beads (Thermo Fisher Scientific). After that, 500 µg of total protein was incubated with 5 µg of antibody or IgG at 4°C overnight. Then, 25 µL protein A/G magnetic beads was added and incubated at 4°C for another 1 hour. After washing, the proteins were eluted with sodium dodecyl sulfate sample buffer and separated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The gel lanes were excised, destained, washed, dried, and dehydrated with acetonitrile. Finally, the peptides were extracted, concentrated, and measured with liquid chromatography with tandem mass spectrometry.

Results

PRMT7 expression is higher in lung cancer tissues than in normal tissues

Previously, gene expression meta-analysis identified *PRMT7* as a candidate gene involved in breast cancer metastasis.[17] Later, Baldwin et al reported the overexpression of *PRMT7* promotes cell invasion in breast cancer.[27] Similarly, Yao et al showed *PRMT7* is expressed at a higher level and mediates the induction of epithelial-to-mesenchymal transition and metastasis in breast carcinoma cells.[18] These studies consistently indicate *PRMT7* as a promising biomarker and therapeutic target for breast cancer. However, not much has been reported on the function of *PRMT7* in the metastasis of NSCLC, which constitutes the primary goal of the current work.

To gain insights into the potential role of *PRMT7* in NSCLC, we analyzed the public data from the online database (Gene Expression across Normal and Tumor tissue), which provides the expression patterns of a specific gene, for example, *PRMT7*, across both normal and cancer tissues of humans on the basis of profiling with Affymetrix U133plus2 platform. It helps to facilitate the identification of cancer outliers overexpressed only in a subset of patients.[22]
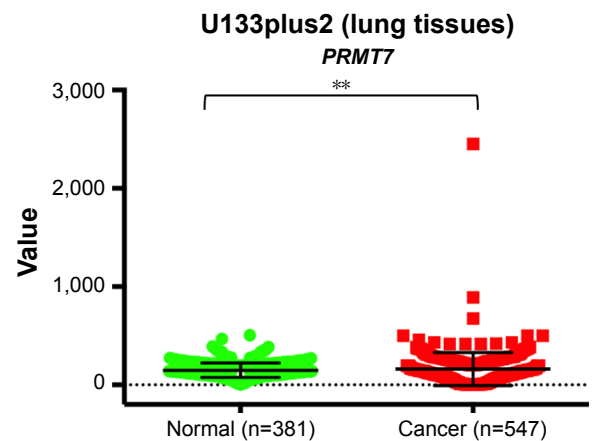

Figure 1 Expression patterns of *PRMT7* in normal and cancerous lung tissues from analysis of U133plus2 dataset (http://medicalgenome.kribb.re.kr/GENT/).
Note: *PRMT7* is significantly upregulated in lung cancer tissues compared to normal controls (**$P<0.01$).

Our results showed that the expression of *PRMT7* was significantly higher in lung cancer tissues than in normal tissues (Figure 1), suggesting the potential role of *PRMT7* in the oncogenesis of lung cancer.

PRMT7 overexpression promotes the invasion and colony formation of NSCLC cells

To investigate the potential roles of *PRMT7* in NSCLC, we constructed *PRMT7*-overexpressed A549 and SPC-A1 cell models with lentiviral transduction method.[28] The expression levels of *PRMT7* were assessed with Western blot analysis in both cell lines, which confirmed significantly upregulated expression of *PRMT7* (Figure 2A).

On this basis, we evaluated the effects of *PRMT7* overexpression on the metastasis phenotype of NSCLC cells. In the Matrigel invasion experiment, our results showed that *PRMT7* overexpression significantly increased the cell invasion of both A549 and SPC-A1 cells compared to the vector controls (Figure 2B), which can be evidenced by the enhanced intensity of crystal violet staining. Consistently, the quantitative results confirmed the above observations, with statistical significance found in the differences ($P<0.05$), as shown in Figure 2C.

We also performed the colony formation assays and found the overexpression of *PRMT7* largely promoted the colony-forming ability in both A549 and SPC-A1 cells compared to the vector controls (Figure 2D), which was further confirmed by the quantification and statistical analysis ($P<0.05$), as shown in Figure 2E.

Together, these results clearly indicated *PRMT7* overexpression was able to contribute to the metastasis phenotype of NSCLC cells, confirming the essential role of *PRMT7* in the
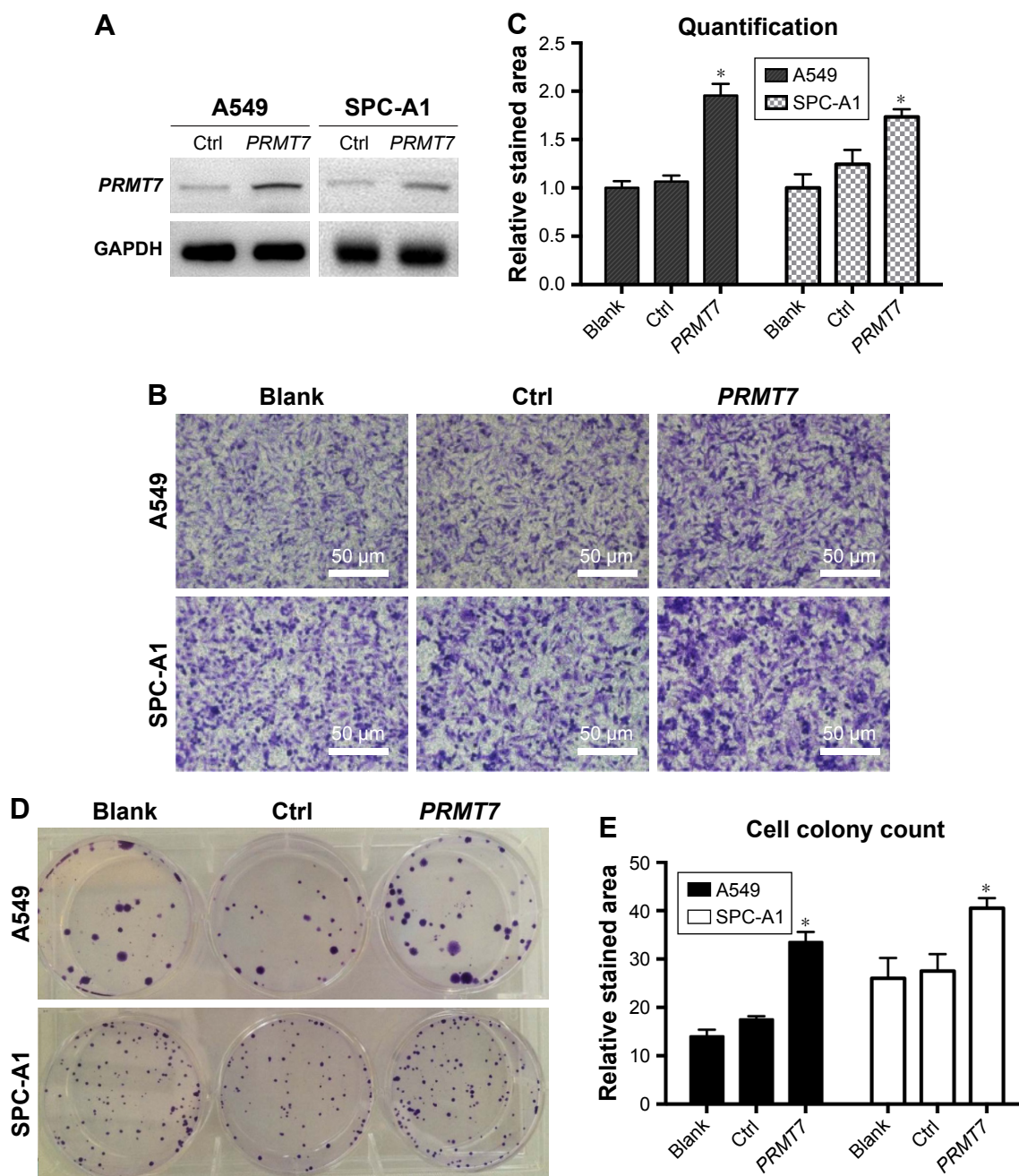
Figure 2 Overexpression of *PRMT7* in A549 and SPC-A1 cells promoted cellular invasion and colony formation.

Notes: (**A**) Expression level of *PRMT7* in A549 and SPC-A1 cells was measured with Western blot analysis. (**B**) Invasion of A549 and SPC-A1 cells was enhanced by *PRMT7* overexpression. Images were collected from a representative experiment. (**C**) Quantitative results of cell invasion. (**D**) Colony formation of A549 and SPC-A1 cells was promoted by *PRMT7* overexpression. Images were collected from a representative experiment. (**E**) Quantitative results of cell colony formation. *$P<0.05$, compared to control group.

Abbreviation: Ctrl, control.

oncogenesis of NSCLC. Our results are in agreement with previous findings that PRMT could promote oncogenesis in cancer.[27]

Exploring *PRMT7*-interacting proteins in both A549 and SPC-A1 cells

On the above basis, we can see *PRMT7* contributes to the metastasis phenotype of NSCLC cells. However, little is

known about the molecular basis of the mechanism. Recently, Jain et al reported the epigenetic control via allosteric regulation of PRMTs,[29] which inspired us to investigate the interacting proteins for *PRMT7*. To this end, we performed co-immunoprecipitation experiments against *PRMT7* in both A549 and SPC-A1 cells, which were followed by silver staining and mass spectrometry analysis. As a result, a collection of 79 candidate proteins were recognized, from which

Table 1 Nineteen in-common proteins shared by both A549 and SPC-A1 cells, which were identified

No.	Protein full name	Protein short name	Score	Mass (Dalton)
1	Desmoplakin	DSP	43	334,021
2	Alpha-actinin-4	ACTN4	648	105,245
3	Neutral alpha-glucosidase AB	GANAB	175	96,441
4	Elongation factor 2	EEF2	166	96,246
5	Desmocollin-1	DSC1	62	101,406
6	78 kDa glucose-regulated protein	HSPA5	4,301	72,402
7	Procollagen galactosyltransferase 1	COLGALT1	554	71,933
8	Endoplasmin	HSP90B1	365	92,696
9	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 3	PLOD3	138	85,302
10	ATP-dependent RNA helicase DDX3X	DDX3X	134	73,597
11	Moesin	MSN	126	67,892
12	Polyadenylate-binding protein 1	PABPC1	96	70,854
13	Protein disulfide-isomerase A4	PDIA4	49	73,229
14	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	DLAT	2,197	69,466
15	X-ray repair cross-complementing protein 6	XRCC6	932	70,084
16	Prelamin-A/C	LMNA	260	74,380
17	Stress-70 protein, mitochondrial	HSPA9	235	73,920
18	Sodium/potassium-transporting ATPase subunit alpha-2 (fragment)	ATP1A2	138	79,827
19	ATP synthase subunit beta (fragment)	ATP5B	50	38,226

19 in-common targets shared by both A549 and SPC-A1 cell lines were selected for further studies (Table 1).

Gene Ontology, Kyoto Encyclopedia of Genes and Genomes pathway, protein binding analysis of the identified proteins

Gene Ontology (GO) annotation was performed on 19 in-common proteins shared by both A549 and SPC-A1 cells. The top eight categories are shown in Table 2. Notably, the term of cell motility got the highest rank, which was followed by acetyl-CoA biosynthesis, proton/hydrogen transport, non-recombinational repair, and double-strand break

repair. Given the experimentally established evidences that tumor cell motility is essential for the progression of cancer metastasis,[30] our results provide insights into the molecular basis underlying the *PRMT7*-promoted metastasis phenotype of NSCLC cells.

We also performed Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and binding protein analysis on the above 19 target proteins. Our results showed that the proteins were primarily involved in prion disease, regulation of actin cytoskeleton, ATP synthesis, pyruvate metabolism, and MAPK signaling pathway (Table 3). Moreover, the 19 *PRMT7*-interacting proteins are likely to bind with APOB, NCF4, PKP2, PXN, PSME3, PECAM1, JUP, and PAIP1 (Table 4). In particular, heat shock 70 kDa protein 5 (*HSPA5*) was found to participate in a wide variety of pathways and protein–protein interactions, suggesting its complex role in regulating cell signaling and metabolism.

Validation of identified *PRMT7*-interacting proteins

To validate the above experimental results, we selected two candidates from 19 in-common proteins for further investigations, including *HSPA5* and *EEF2*. Our selection was mainly based on the previous reports that both of them are involved in cancer metastasis.[31,32] For example, Chen et al found the expression of *HSPA5* was positively correlated with the metastatic events in triple-negative breast cancer patients.[31] Moreover, downregulation of *HSPA5* significantly abolished cancer metastasis in the xenograft model.[31] Song et al revealed that the overexpression of *EEF2* increased the cell migration and invasion in lung squamous cell carcinoma.[32] On these bases, we were encouraged to investigate the effects of *HSPA5* and *EEF2* on the oncogenic function of *PRMT7*.

We performed standard co-immunoprecipitation experiment to precipitate *PRMT7* complex with anti-*PRMT7* antibody in both A549 and SPC-A1 cells. Then, we detected the target proteins in the co-precipitated products with

Table 2 GO analysis of 19 in-common proteins shared by both A549 and SPC-A1 cells

No.	GO terms	Genes	*P*-value
1	Cell motility	ACTN4, ATP1A2, MSN	0.0008
2	Acetyl-CoA biosynthesis	DLAT	0.001
3	Proton transport	ATP1A2, ATP5B	0.002
4	Hydrogen transport	ATP1A2, ATP5B	0.002
5	ATP hydrolysis coupled proton transport	ATP1A2	0.003
6	Non-recombinational repair	G22P1	0.003
7	Double-strand break repair via nonhomologous end joining	G22P1	0.003
8	Energy-coupled proton transport against electrochemical gradient	ATP1A2	0.005

Abbreviation: GO, Gene Ontology.

Table 3 KEGG pathway analysis of 19 in-common proteins shared by both A549 and SPC-A1 cells

No.	KEGG pathway	Genes	*P*-value
1	Prion disease	*HSPA5*	0.004
2	Regulation of actin cytoskeleton	*ACTN4, MSN*	0.01
3	ATP synthesis	*ATP5B*	0.01
4	Pyruvate metabolism	*DLAT*	0.01
5	MAPK signaling pathway	*HSPA5, HSPA9B*	0.02

Abbreviation: KEGG, Kyoto Encyclopedia of Genes and Genomes.

Western blot analysis. Our results showed that the *HSPA5* and *EEF2* bands were clearly present in the immunoblots of *PRMT7* co-precipitants in both A549 and SPC-A1 cells (Figure 3). In contrast, only weak band of similar molecular weight was detected in the control precipitants (Figure 3). These results validated that *PRMT7* interacts with *HSPA5* and *EEF2* directly in both NSCLC cell lines.

HSPA5 and *EEF2* mediated *PRMT7*-induced metastasis phenotype in NSCLC cells

We further investigated the function of *HSPA5* and *EEF2* in mediating *PRMT7*-induced metastasis in NSCLC. Based on the above results, it was found that *PRMT7* overexpression was able to promote invasion in both A549 and SPC-A1 cell lines. We screened a collection of siRNAs by transfecting them into NSCLC cells to evaluate the knockdown effect against a specific gene (*HSPA5* or *EEF2*). The relative mRNA expressions of both target genes are shown in Figure 4A. The siRNAs with significant effect and balanced performance between both cell lines were selected for the following experiment (*HSPA5*: si-2082 and *EEF2*: si-683).

Afterward, we transfected the selected siRNAs into *PRMT7*-overexpressed A549 and SPC-A1 cells and assessed their effects on the cell invasion with Matrigel invasion assays. From our results, it was found that compared to the vector control, knockdown of *HSPA5* or *EEF2* gene significantly reduced *PRMT7*-promoted invasion of NSCLC

cells, as evidenced by the decreased intensity of crystal violet staining in both A549 and SPC-A1 cells (Figure 4B). Quantitative results further confirmed our observations with a statistically significant difference ($P<0.05$; Figure 4C). Collectively, these results suggested that *HSPA5* and *EEF2* may mediate *PRMT7*-induced NSCLC cell invasion through directly interacting with *PRMT7*.

Discussion

PRMTs participate in a wide variety of physiologic and pathogenetic processes.[7,33,34] As the unique member characterized by both type II and III enzyme activity, *PRMT7* was found to be a metastasis promoter gene in breast cancer.[17,18,27] In the present work, we found *PRMT7* expression was significantly increased in the lung cancer tissues by analyzing the target gene expression patterns in $>34,000$ human samples. Moreover, overexpression of *PRMT7* in two different NSCLC cell lines (A549 and SPC-A1) was found to promote the cell invasion and colony formation consistently, suggesting *PRMT7* plays an important role in promoting the metastasis phenotype of NSCLC cells.

A recent report published in 2017 revealed the new mechanism of epigenetic control via allosteric regulation of PRMTs.[29] In order to explore the molecular basis underlying the contribution of *PRMT7* to the cell invasion in NSCLC, we performed co-immunoprecipitation experiments and mass spectrometry to explore the potential interacting proteins for *PRMT7*. As a result, 79 candidates were identified and 19 in-common genes shared by both A549 and SPC-A1 cell lines were highlighted, which constitute the focus of following studies. To our knowledge, there is no report focusing on the *PRMT7*-interacting regulation proteins in NSCLC. Our results laid the basis for the investigation of mechanism as well as target-based drug discovery.

Bioinformatic analysis, for example, GO annotation, revealed the close relationship between the above identified proteins and cell motility, which is known to contribute to

Table 4 Binding protein analysis of 19 in-common proteins shared by both A549 and SPC-A1 cells

| No.	Binding proteins	Genes	*P*-value
1	APOB: apolipoprotein B (including Ag(x) antigen)	*ERP70, HSPA5, TRA1*	<0.0001
2	NCF4: neutrophil cytosolic factor 4, 40 kDa	*G22P1, MSN*	<0.0001
3	PKP2: plakophilin 2	*DSC1, DSP*	0.0004
4	PXN: paxillin	*G22P1, HSPA5, LMNA, PABPC1*	0.0006
5	PSME3: proteasome (prosome, macropain) activator subunit 3	*ATP5B, HSPA5*	0.0006
6	PECAM1: platelet/endothelial cell adhesion molecule (CD31)	*DSP, G22P1*	0.002
7	JUP: junction plakoglobin	*DSC1, DSP*	0.005
8	PAIP1: poly(A) binding protein interacting protein 1	*PABPC1*	0.005
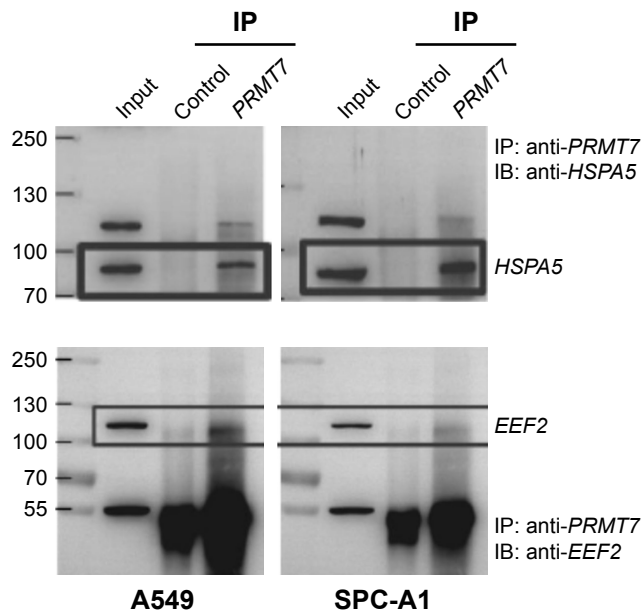
Figure 3 Validation of the interactions between *PRMT7* and *HSPA5*, *EEF2* by co-immunoprecipitation and Western blot analysis in A549 and SPC-A1 cells.
Abbreviations: IP, immunoprecipitation; IB, immunoblot.

Collectively, our results provide insights into the molecular basis underlying the *PRMT7*-promoted metastasis phenotype of NSCLC cells.

Among 19 candidates, we selected two genes (*HSPA5* and *EEF2*) for further studies. According to previous reports, both proteins are shown to be highly involved in the regulation of cancer metastasis. For instance, *HSPA5* expression was found to be positively correlated with metastasis in triple-negative breast cancer patients, and downregulation of *HSPA5* significantly abolished cancer metastasis in a xenograft tumor model.[31] In addition, expression of *EEF2* in cells increased the capabilities of migration and invasion of cancer cells.[32] As expected, the interactions between *PRMT7* and two candidate proteins (*HSPA5* and *EEF2*) were confirmed with co-immunoprecipitation and Western blot analysis, which suggests the reliability of our experimental protocols. Therefore, it is worth investigating the other 17 identified candidate proteins in future studies (Table 1), which constitutes the focus of our following work.

To assess the effects of both *HSPA5* and *EEF2* proteins on *PRMT7*-induced metastasis in NSCLC, we constructed A549 and SPC-A1 cell models bearing both *PRMT7* gene overexpression and *HSPA5*/*EEF2* gene knockdown. The observation that cell invasion was significantly reduced in both cell lines suggested that these two proteins might serve as essential regulators in the promotion of metastasis
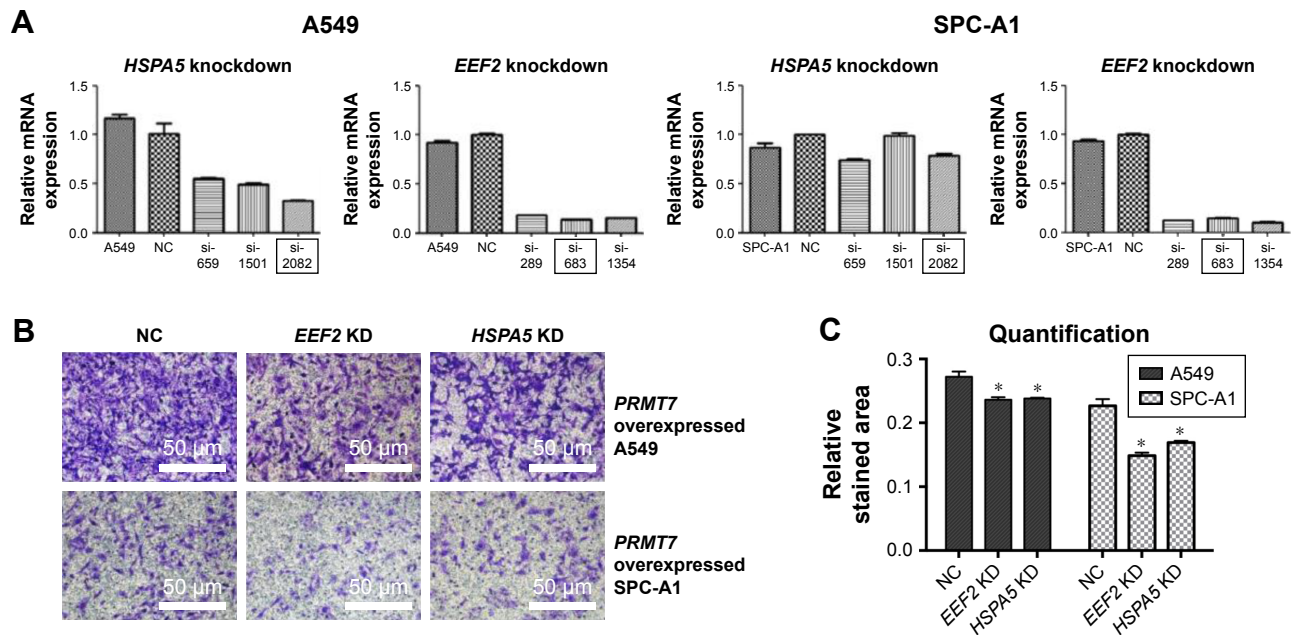
the progression of cancer metastasis.[30] KEGG pathway and binding protein analysis consistently indicate the complex role of *HSPA5*, which participates in a wide variety of signaling and protein–protein interactions. *HSPA5*, a member of the heat shock protein 70 (HSP70) family, is involved in protein folding and assembly in the endoplasmic reticulum.

Figure 4 Cell invasion was reduced by gene knockdown of *HSPA5* or *EEF2*.
Notes: (**A**) mRNA levels of *HSPA5* and *EEF2* were measured with real-time PCR. (**B**) The invasion of A549 and SPC-A1 cells bearing *PRMT7* overexpression was reduced by the gene knockdown of *HSPA5* or *EEF2*. (**C**) Quantification of cell invasion in A549 and SPC-A1 cells. *$P < 0.05$, compared to the control group.
Abbreviations: PCR, polymerase chain reaction; NC, normal control.

by *PRMT7*. In-depth study of the relationship between *HSPA5*, *EEF2*, and *PRMT7* will help to clarify the mechanism of *PRMT7* in NSCLC metastasis, which also constitutes the main aim of our work.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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