LncRNA PSMG3-AS1 Downregulates miR-340 through Methylation to Upregulate ROCK1 and Promote Cell Invasion and Migration in Non-small Cell Lung Cancer

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Abstract

Background: LncRNA PSMG3-AS1 plays oncogenic role in breast cancer. However, its role in non-small cell lung cancer (NSCLC) is hardly known. We then studied the role of PSMG3-AS1 in NSCLC.

Methods: RT-qPCR was performed to determine the expression of PSMG3-AS1 in NSCLC and non-tumor tissues from 60 NSCLC patients. A survival analysis was carried out to visit patients for 5 years to study the role PSMG3-AS1 in prediction the survival of NSCLC. NSCLC cells were overexpressed with miR-340 or PSMG3-AS1 to analyze the crosstalk between PSMG3-AS1 and miR-340. MSP was performed to analyze the methylation of miR-340 miRNA gene. The invasion and migration abilities of cells were determined by Transwell assays.

Results: PSMG3-AS1 was highly expressed in NSCLC and was closely correlated poor survival. PSMG3-AS1 and miR340 were inversely correlated. In NSCLC cells, PSMG3-AS1 decreased the expression of miR-340 and increased methylation of miR-340 gene. However, miR-340 overexpression did not significantly affect the expression of PSMG3-AS1. In addition, PSMG3-AS1 overexpression resulted in upregulated expression of ROCK1. PSMG3-AS1 and ROCK1 overexpression increased cell invasion and migration rates. MiR-340 overexpression suppressed cell behaviors and inhibited the role of PSMG3-AS1.

Conclusions: PSMG3-AS1 may downregulate miR-340 through methylation to upregulate ROCK1 and promote cell invasion and migration in NSCLC.

1. Introduction

Lung cancer is the most common cancer, mainly owing to its high prevalence across the world and the extremely aggressive nature(1, 2). The latest GLOBOCAN statistical data illustrated that lung cancer in 2018 accounted for 11.6% of all new cases and 18.4% of all cancer deaths(3). Smoking has been characterized as the major risk factor for lung cancer(4). Quitting smoking requires intensive interference of daily life and is not practical in many cases(5). In addition, never-smokers may also develop lung cancer(6, 7). Therefore, novel preventive and treatment approaches are needed.

Molecular alterations participate in the initiation and progression of lung cancer(8, 9). In effects, some molecular players with critical functions in lung cancer are likely potential targets to treat cancers(10). With the capacity of gene expression regulation, lncRNAs are crucial players in cancer biology(11). Therefore, lncRNAs may also serve as potential targets for targeted therapy. LncRNA PSMG3-AS1 promotes breast cancer, while its role in non-small cell lung cancer (NSCLC) is unknown. By analyzing our preliminary deep sequencing data we observed the close correlation between PSMG3-AS1 and miR-340, which mainly targets ROCK1 to participate in cancers(12), is unknown. We then studied the crosstalk between PSMG3-AS1, miR-340 and ROCK1 in NSCLC.

2. Materials And Methods

2.1 NSCLC patients and tissues

The 60 NSCLC patients (40 males and 20 females) were subjected to biopsies for the collection of paired NSCLC and non-tumor tissues. All these patients were diagnosed for the first time at Suzhou Hospital Affiliated to Anhui Medical University (Suzhou Municipal Hospital, Ethics Committee approved this study).. Age of these patients ranged from 40 years to 69 years (54.7 ± 5.9 years). All tissue samples were confirmed by histopathological exam, followed by RNA isolation. All patients signed informed consent.

2.2 Follow-up

The 60 NSCLC patients (23 cases and 37 cases at stage I/II or III/IV)) were treated with chemotherapy, radiotherapy, surgical resection and their combinations according to their health conditions and cancer stages. The 60 patients were visited every month for 5 years to monitor their survival. The 60 patients were excluded from the ones died of causes unrelated to NSCLC.

2.3 NSCLC cells and transfections

Human NSCLC cell line H1993 (ATCC, USA) was used and cells were cultivated following instructions from ATCC..

Cells were transfected with pcDNA3.1-PSMG3-AS1 vector, pcDNA3.1-ROCK1 vector or miR-340 mimic through transfections mediated by Lipofectamine 2000 (Invitrogen). Negative control experiments were performed by transfecting H1993 cells with NC miRNA or empty pcDNA3.1 vector. Control cells were untransfected cells. Subsequent experiments were performed 48h later.

2.4 RNA preparations

RNAzol (Invitrogen) was used to isolate RNAs from H1993 cells and paired tissue samples, followed by using DNase I (Invitrogen) to completely remove genomic DNAs. RNA purity was determined based on OD260/280 ratio.

2.5 RT-qPCR

RNA samples (OD260/280 ratio of 2.0) were subjected to cDNA preparations using SSRT III system (Invitrogen). All qPCRs were performed using cDNA samples as template to determine the expression of PSMG3-AS1 and ROCK1 mRNA with GAPDH as internal control. All-in-One™ miRNA qRT-PCR Detection Kit (Genecopoeia) was used to study mature miR-340 expression. The method of $2^{-\Delta\Delta C_T}$ was used to normalize Ct values.

2.6 Methylation-specific PCR (MSP)

DNA samples were isolated from cells transfected with empty pcDNA3.1 vector or PSMG3-AS1 expression vector with Monarch® Genomic DNA Purification Kit (NEB). EZ DNA Methylation Lighting Kit (ZYMO research) was used to convert DNA samples and methylation of miR-340 gene was analyzed by RT-qPCR.

2.7 Western blot

RIPA solution (Invitrogen) and BCA assay (Invitrogen) were used to isolate and quantify protein samples. Denatured protein samples were separated by SDS-PAGE gels (10%) and gel transfer was performed using PVDF membranes. Membranes were blocked in FBS containing 5% non-fat milk. After that, primary antibodies of GAPDH (ab9483, Abcam) and ROCK1 (ab97592, Abcam) were incubate with membranes at 4°C for 18h. After that, at room temperature, membranes were further incubated with HRP (IgG) (ab6721, Abcam) for 2h. Signals were produced using ECL (Invitrogen) and Quantity One software was used for data quantification.

2.8 Transwell assays

H1993 cells were subjected to the analysis of invasion and migration abilities using Transwell filters (8 µm, Dojindo). Before invasion assay, membranes were coated with Matrigel (Dojindo) at 37°C for 10h, while migration assays were performed using uncoated membranes. Cells in fresh serum-free medium were transferred to the upper Transwell chamber with 5000 cells per well, while with the lower chamber was filled with 20% FBS medium. Under the aforementioned conditions, cells were cultivated for 12h, followed by crystal violet (0.1%) staining of the lower surface of the membranes. Cells were observed under a light microscope.

2.9 Statistical analysis

Unpaired t test and ANOVA Tukey's test were used to compared paired samples and multiple groups, respectively. Survival analysis was performed by dividing the 60 patients into high and low PSMG3-AS1 level groups (n = 30), followed by the plotting of survival curves for both groups and comparison of curves by log-rank test. $P < 0.05$ was deemed statistically significant.

3. Results

3.1 PSMG3-AS1 was highly expressed in NSCLC and was closely correlated with poor survival

PSMG3-AS1 expression in paired NSCLC and non-tumor tissues from 60 NSCLC patients was analyzed by RT-qPCR. Our data illustrated that NSCLC tissues exhibited significantly higher levels of PSMG3-AS1 expression (Fig. 1A, $p < 0.001$). Survival curve data illustrated that the overall survival rate was significantly higher in low PSMG3-AS1 level group in comparison to high PSMG3-AS1 level group (Fig. 1B).

3.2 PSMG3-AS1 decreased miR-340 expression through methylation

Expression of miR-340 was determined by RT-qPCR. Linear regression analysis showed that PSMG3-AS1 and miR340 were inversely correlated across the 60 NSCLC tissue samples (Fig. 2A), indicating the potential interaction between them in NSCLC. To explore the interaction between them, H1993 cells were overexpressed with PSMG3-AS1 or miR-340 (Fig. 2B, $p < 0.05$). PSMG3-AS1 expression vector showed significantly downregulated miR-340, while cells with miR-340 mimic transfection exhibited no significant changes in expression levels of PSMG3-AS1 (Fig. 2C). MSP analysis revealed that PSMG3-AS1 expression significantly increased methylation of miR-340 gene (Fig. 2D).

3.3 PSMG3-AS1 overexpression resulted in upregulated expression of ROCK1

ROCK1 is a validated target of miR-340. In this study, the effects of PSMG3-AS1 and miR-340 overexpression on the expression of ROCK1 were analyzed by RT-qPCR (Fig. 3A) and Western blot (Fig. 3B). Our data illustrated that miR-340 decreased the expression of ROCK1 expression, while PSMG3-AS1 increased ROCK1 expression and suppressed the role of miR-340 ($p < 0.05$).

3.4 PSMG3-AS1 regulates miR-340 and ROCK1 to promote cell invasion and migration

Transwell assays were carried out to study the roles of PSMG3-AS1, miR-340 and ROCK1 in regulating the invasion (Fig. 4A) and migration (Fig. 4B) of H1993 cells. It was observed that PSMG3-AS1 and ROCK1 overexpression resulted in increased cell invasion and migration rates. MiR-340 overexpression played an opposite role and reduced the effects of PSMG3-AS1 overexpression ($p < 0.05$).

4. Discussion

The role of PSMG3-AS1, miR-340 and ROCK1 in NSCLC and crosstalk between them were studied. Our data illustrated that PSMG3-AS1 was highly expressed in NSCLC and it may increase miR-340 RNA gene methylation to upregulate ROCK1, thereby promoting NSCLC.

PSMG3-AS1 promotes breast cancer(13). It was reported that PSMG3-AS1 was upregulated in breast cancer and promoted cancer cell migration and proliferation by sponging miR-143-3p(12). We first reported the upregulation of PSMG3-AS1 in NSCLC. Moreover, increased cancer cell invasion and migration were observed after PSMG3-AS1 overexpression. Therefore, PSMG3-AS1 may play oncogenic roles in NSCLC.

Most NSCLC patients were diagnosed at late stages and only less than 20% of NSCLC patients can survive for 5 years after the initial diagnosis(14, 15). The improvement of early diagnosis of NSCLC is limited by the lack of effective tumor biomarkers, and this situation is unlikely to be changed in near future(16). Therefore, accurate prognosis may assist the selection of therapeutic approaches. We showed that PSMG3-AS1 expression in NSCLC tissues was correlated with NSCLC patients' survival.

MiR-340 plays a role as tumor suppressor in many cancers including NSCLC(17). MiR-340 was downregulated in NSCLC and targeted CDK1 to suppress cell proliferation(17). In another study, Zhou et al. reported that miR-340 could target ROCK1 to suppress tumor metastasis and growth in osteosarcoma(12). In this study we showed that miR-340 may also target ROCK1 in NSCLC to suppress cell invasion and migration. Therefore, miR-340 may target multiple oncogenes in NSCLC to play tumor suppressive roles.

In a recent study miR-340 was reported to be sponged by a lncRNA in lung cancer (18). Interestingly, we showed that PSMG3-AS1 could downregulate miR-340 by increasing the methylation of miR-340 gene. Therefore, this study characterized a novel PSMG3-AS1/miR-340/ROCK1 pathway in NSCLC.

5. Conclusion

PSMG3-AS1 is highly expressed in NSCLC and may upregulate ROCK1 by downregulating miR-340 through methylation to promote NSCLC cell invasion and migration.

Declarations

Acknowledgments

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Ethical Approval and Consent to participate

Informed consent was obtained from all individual participants included in the study. All procedures were approved by Suzhou Hospital Affiliated to Anhui Medical University (Suzhou Municipal Hospital) Ethics Committee. Procedures operated in this research were completed in keeping with the standards set out in the Announcement of Helsinki and laboratory guidelines of research in China.

Consent to publish

Not applicable.

Availability of supporting data

The data that support the findings of this study are available on request from the corresponding author: *Qingsheng Kan, Department of Oncology, Suzhou Hospital Affiliated to Anhui Medical University (Suzhou Municipal Hospital), No. 299 Bianhe Middle Road, Suzhou City, Anhui Province, 234000, PR. China.

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The data are not publicly available due to their containing information that could compromise the privacy of research participants.

Competing interests

All other authors have no conflicts of interest.

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Authors' contributions

Xiyong Wang, Qingsheng Kan: study concepts, literature research, clinical studies, data analysis, experimental studies, manuscript writing and review; Yang Yang: study design, literature research, experimental studies and manuscript editing; Yu Dai: definition of intellectual content, clinical studies, data acquisition and statistical analysis; Hongming Zhang: data acquisition, manuscript preparation and data analysis; Honglin Xia: data acquisition and statistical analysis.

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Figures

Figure 1

PSMG3-AS1 was highly expressed in NSCLC and was closely correlated with poor survival PSMG3-AS1 expression in paired NSCLC and non-tumor tissues from 60 NSCLC patients was analyzed by RT-qPCR. Expression data of PSMG3-AS1 and miR-340 in paired NSCLC and non-tumor tissues was analyzed in three replicates (A), ***, $p < 0.001$. Survival analysis was performed through the aforementioned methods (B).

Figure 2

PSMG3-AS1 decreased miR-340 expression through methylation Expression of miR-340 in NSCLC tissues from 60 NSCLC patients was analyzed by RT-qPCR. Linear regression analysis showed that PSMG3-AS1 and miR340 were inversely correlated across the 60 NSCLC tissue samples (A). To explore the interaction between them, H1993 cells were overexpressed with PSMG3-AS1 and miR-340 (B). The effects of PSMG3-AS1 and miR-340 overexpression on the expression of each other were also analyzed by RT-qPCR (C). MSP was performed to analyze the effects of PSMG3-AS1 overexpression on miR-340 expression (D). *,p<0.05.

Figure 3

PSMG3-AS1 overexpression resulted in upregulated expression of ROCK1. ROCK1 is a validated target of miR-340. In this study, the effects of PSMG3-AS1 and miR-340 overexpression on the expression of ROCK1 were analyzed by RT-qPCR (A) and Western blot (B). *, $p < 0.05$.

Figure 4

PSMG3-AS1 regulates miR-340 and ROCK1 to promote cell invasion and migration. Transwell assays were performed to analyze the roles of PSMG3-AS1, miR-340 and ROCK1 in regulating the invasion (A) and migration (B) of H1993 cells. *, $p < 0.05$.