miR-143-3p Targets IncRNA PSMG3-AS1 to Inhibit the Proliferation of Hepatocellular Carcinoma Cells

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Introduction: The molecular pathogenesis of liver cancer remains unclear; some ncRNAs have been considered as potential drug targets for cancer treatment. IncRNA PSMG3-AS1 has been reported to promote breast cancer, while its role in hepatocellular carcinoma (HCC) is unknown.

Methods: Bioinformatics analysis was conducted to investigate the relationship between miR-143-3p and PSMG3-AS1. RT-qPCR was used to detect the expression levels of miR-143-3p and PSMG3-AS1 and the correlation between them in HCC. The survival curve was used to analyze the effect of PSMG3-AS1 on the prognosis of liver cancer. RT-qPCR was used to detect the effect of different concentration gradients of miR-143-3p on PSMG3-AS1. CCK8 and clone formation experiments were used to examine the role of miR-143-3p and PSMG3-AS1 in regulating the proliferation of SNU-182 and SNU-398 cells.

Results: Our preliminary bioinformatics analysis showed that miR-143-3p can target PSMG3-AS1. We, therefore, analyzed the interaction between PSMG3-AS1 and miR-143-3p in HCC. We found that PSMG3-AS1 was upregulated, while miR-143-3p was downregulated in HCC. The expression levels of PSMG3-AS1 and miR-143-3p were closely and inversely correlated with each other. High expression levels of PSMG3-AS1 predicted poor survival. In HCC cells, overexpression of PSMG3-AS1 led to increased proliferation rates. Overexpression of miR-143-3p played an opposite role and reversed the effect of overexpression of PSMG3-AS1.

Discussions: miR-143-3p may target PSMG3-AS1 to inhibit the proliferation of HCC cells.

Keywords: hepatocellular carcinoma, IncRNA PSMG3-AS1, miR-143-3p, proliferation

Introduction
As a type of malignancy contains intrahepatic cholangiocarcinoma (ICC) and hepatocellular carcinoma (HCC), liver cancer is a major burden on the public health.1 Liver cancer in 2018 alone affected 841,080 new cases (4.7% of all new cancer cases), which ranked the 7th most common type of malignancies.2 In contrast, due to its extreme aggressive nature, liver cancer caused 781,631 deaths (8.2% of all cancer deaths), which made it the 3rd most common cause of cancer-related deaths.3 Liver cancer is mainly caused by the use of tobacco and alcohol as well as the infections of hepatitis B virus (HBV) and C virus (HCV).4,5 Although our understanding of the mechanism of HBV and HCV infections has been significantly increased during the past decade,5,6 the molecular pathogenesis of liver cancer remains unclear.
The development and progression of liver cancer involves multiple signaling pathways. Functional analyses of the signaling pathways involved in this disease provide novel insights into the development of targeted therapies for cancer treatment. Although non-coding RNAs (ncRNAs), such as microRNAs (miRNAs) and long (> 200 nt) ncRNAs (lncRNAs), are not involved in protein coding, regulate the expression of cancer-related genes to promote or suppress cancer development. In effect, some ncRNAs have been considered as potential drug targets for cancer treatment. However, the functions of most ncRNAs in cancer biology remain unclear. PSMG3-AS1 is a novel tumor-suppressive lncRNA characterized in breast cancer, while its role in HCC, a major subtype of liver, remains unknown. Our preliminary bioinformatics analysis showed that PSMG3-AS1 may be targeted by miR-142-3p, which is a well-characterized tumor suppressor. This study was therefore carried out to investigate the interaction between PSMG3-AS1 and miR-142-3p in HCC.

Patients and Methods

Patients, Treatment and Follow-Up

A total of 66 HCC patients (40 males and 26 females, 43 to 69 years old, mean age 55.1 ± 6.3 years old) admitted at the First Affiliated Hospital of Anhui Medical University between May 2012 and May 2014 were enrolled. All HCC patients were new cases diagnosed by histopathological exams (Figure S1). No therapy was initiated, and patients with other clinical disorders were excluded. According to their medical record, 20 cases were HBV positive only, 17 cases were HCV positive only, 13 cases were positive for both HBV and HCV, and 16 cases were negative for both HBV and HCV. Treatments were determined based on their AJCC stages. The 68 patients included 9 stage I, 10 stage II, 24 stage III and 23 cases stage IV cases. Surgical resection, chemotheraphy, radiotherapy, targeted therapy or combined therapies were performed. All patients signed the written informed consent. All patients were followed up for 5 years after admission. All patients completed the 5-year follow-up. Their survival conditions were recorded and used for survival analysis. This study was approved by the Ethics Community of the aforementioned hospital and was conducted in accordance with the Declaration of Helsinki.

Tissues and HCC Cells

All patients were diagnosed by histopathological biopsy. During biopsy, both HCC and adjacent non-tumor tissues (within 3 cm around tumors) were collected from each patient. All tissue samples were tested by histopathological analysis and were stored in liquid nitrogen before use. SNU-182 and SNU-398 cells obtained from ATCC (USA) were used to perform in vitro experiments. Cell culture medium was composed of 10% FBS and 90% RPMI 1640 medium. Cells were cultivated in a 5% CO2 incubator at 37 °C with 95% humidity.

RNA Preparations and RT-qPCR

Total RNAs were isolated from biopsies and cultivated cells using RNAzol (Sigma-Aldrich). All RNA samples were digested with DNase I (Sigma-Aldrich) to remove genomic DNAs. RNA concentrations were measured by NanoDrop 2000 Spectrophotometer (Thermo Scientific). RNA samples with satisfactory qualities were subjected to reverse transcriptions using QuantiTect Reverse Transcription Kit (QIAGEN) to synthesize cDNA samples. With cDNA samples as template, qPCR reactions were performed using SYBR® Green Quantitative RT-qPCR Kit (Sigma-Aldrich). The expression levels of PSMG3-AS1 were measured with 18S rRNA as endogenous control. The expression levels of mature miR-143-3p were measured using All-in-One miRNA qRT-PCR Detection Kit (Genetopoeia). U6 was used as the endogenous control of miR-143-3p. PCR reactions were performed in triplicate. Expression levels were normalized using 2^−ΔΔCt method. Primer sequences were as following: 5'-GAAG CAGAAACCAACGCACAG-3' (forward) and 5'-GCATAA TCCAATCCCCTCAAGAA-3' (reverse) for PSMG3-AS1; 5' CTGGCGTTGAGATGAGCAC-3' (forward) and reverse, 5'-CAGAGCCAGGGTCCGAGGTA-3' (reverse) for miR-143-3p.

Cell Transfection

Expression vector of PSMG3-AS1 was constructed using pcDNA 3.1 (Sigma-Aldrich) as the backbone. miR-143-3p mimic and negative control (NC) miRNA were purchased from Sigma-Aldrich. SNU-182 and SNU-398 cells were collected at about 80% confluence and counted, followed by transfecting 10 nM vector or 10~100 nM siRNA into 10^5 cells using lipofectamine 2000 (Invitrogen). Control (C) cells in all transfections were cells without transfections. NC cells were empty vector- or NC miRNA-transfected cells. Subsequent experiments were performed 48 h post-transfection.

Cell Proliferation Assay

Cell Counting Kit-8 kit (CCK-8, Dojindo) was used to perform cell proliferation assays on SNU-182 and SNU-398
cells collected at 48 h post-transfection. In brief, 3000 cells were transferred to each well of a 96-well cell culture plate. Cells were cultivated under aforementioned conditions, and cell collections were performed every 24 h for a total of 4 days. At 4 h before cell collection, CCK-8 solution was added into each well to reach 10% final concentration. After cell collection, OD values were measured at 450 nm.

Colony Formation Assay
For colony formation assay, the transfected cells were seeded into 6-well plates (4000 cells/well) and incubated in DMEM with 10% FBS. After 2 weeks, cells were fixed by methanol and stained by 0.1% crystal violet. The colony numbers were then counted.

Statistical Analysis
Three biological replicates were included in each experiment. Mean ± SEM values were used to express all data. Paired t-test was used to compare non-tumor and HCC tissues. ANOVA (one-way) and Tukey’s test were used to compare differences among multiple groups. Spearman’s rank correlation coefficient was used for correlation analysis. Survival analysis was performed by dividing the 66 HCC patients into high and low PSMG3-AS1 level groups (n = 33). Survival curves were plotted using GraphPad Prism 6 software and compared by Log-rank test. \( P < 0.05 \) was statistically significant.

Results
The Expression of PSMG3-AS1 and miR-143-3p Was Altered in HCC
The expression levels of PSMG3-AS1 and miR-143-3p in both HCC and non-tumor tissues were measured by RT-qPCR assays. Compared to non-tumor tissues, the expression levels of PSMG3-AS1 were significantly higher in HCC tissues (Figure 1A, \( P < 0.001 \)). In contrast, the expression levels of miR-143-3p were significantly lower in HCC tissues than that in non-tumor tissues (Figure 1B, \( P < 0.001 \)). It is worth noting that the expression levels of PSMG3-AS1 and miR-143-3p were not significantly different among different HBV and HCV infection groups.

(miR-143-3p May Downregulate PSMG3-AS1 by Direct Targeting)
The interaction between miR-143-3p and PSMG3-AS1 was predicted by IntaRNA.\(^{14}\) It was observed that miR-143-3p

![Figure 1](https://example.com/figure1.png) The expression of PSMG3-AS1 and miR-143-3p were altered in HCC. The expression levels of PSMG3-AS1 (A) and miR-143-3p (B) in both HCC and non-tumor tissues were measured by performing RT-qPCR assays. PCR reactions were performed in 3 replicates and mean values were presented and compared. ***\( P < 0.05 \).
might target PSMG3-AS1 (Figure 4A). To confirm this, SNU-182 and SNU-398 cells were transfected with miR-143-3p mimic or PSMG3-AS1 expression vector, followed by the confirmation of the overexpression of miR-143-3p and PSMG3-AS1 by RT-qPCR (Figure 4B, \( p < 0.05 \)). Compared to C and NC groups, overexpression of miR-143-3p led to significantly downregulated PSMG3-AS1 (Figure 4C, \( p < 0.05 \)). In contrast, overexpression of PSMG3-AS1 did not affect the expression of miR-143-3p (Figure 4D).

miR-143-3p Downregulates PSMG3-AS1 to Inhibit Cell Proliferation

CCK-8 analysis (Figure 5A) and clone formation experiments (Figure 5B and C) were conducted to explore the role of miR-143-3p and PSMG3-AS1 in regulating the proliferation of SNU-182 and SNU-398 cells. The cell proliferation rate of the PSMG3-AS1 group increased significantly, while the miR-143-3p group decreased. Compared with group C, there was no significant difference in cell proliferation between PSMG3-AS1 and miR-143-3p cotransfection groups (Figure 5, \( p < 0.05 \)). This shows that miR-143-3p and PSMG3-AS1 play the opposite role and reduce the effect of PSMG3-AS1 overexpression on cell proliferation.

Discussion

Increasing evidence has shown that long non-coding RNAs (lncRNAs) play important roles in tumor progression. In this study, we analyzed the interaction between miR-143-3p and PSMG3-AS1 in HCC. We found that miR-143-3p and PSMG3-AS1 were both dysregulated in liver cancer and miR-143-3p may target PSMG3-AS1 to suppress HCC cell proliferation.

The functionality of PSMG3-AS1 has only been explored in breast cancer.\(^{12}\) It was reported that PSMG3-AS1 was downregulated in breast cancer and may sponge miR-143-3p to promote the migration and proliferation of cancer cells.\(^{12}\) This study is the first to report the upregulation of PSMG3-AS1 in HCC. Moreover, overexpression of PSMG3-AS1 in HCC cells led to decreased proliferation of HCC cells. Therefore, our study proved that PSMG3-AS1 was also likely an oncogenic lncRNA in HCC.

More than 50% of HCC patients diagnosed at an early stage can live longer than 5 years after the initial diagnosis.\(^{15}\) However, most HCC patients are diagnosed at advanced stages, which lack radical treatment. As a consequence, the survival rate of most HCC patients remains low. In this study, we proved that high expression levels of PSMG3-AS1 were closely correlated with poor survival of HCC patients. Therefore, measuring the
expression levels of PSMG3-AS1 before treatment may be used to predict the survival of HCC patients and guide the selection of therapeutic approaches. The analysis of the lncRNA co-expression module of ovarian cancer found that 16 lncRNAs including PSMG3-AS1 were significantly associated with the pathological diagnosis, lymphatic vessel infiltration, tissue source site and the age of patients with vascular infiltration. PSMG3-AS1 may be related to the prognosis of ovarian cancer. However, the prognostic accuracy remains to be further tested.

miR-142-3p has been proven as a tumor-suppressive miRNA in multiple types of malignancy. In osteosarcoma, miR-143-3p can inhibit the proliferation, migration and invasion of osteosarcoma by targeting FOSL2. In breast cancer, miR-143-3p can inhibit the proliferation, cell migration and invasion of human breast cancer cells by regulating the expression of MAPK7. A recent study reported that miR-142-3p is downregulated in HCC and its overexpression can inhibit cell proliferation in HCC. Our study confirmed its downregulation in HCC and its inhibitory role in regulating HCC cell proliferation. We proved that, besides protein coding genes, such as Bach-1 and LDHA, miR-142-3p can also target an lncRNA, which is PSMG3-AS1, to suppress cancer development. However, other mechanisms may also exist, and more studies are still needed.
MiR-143-3p downregulates PSMG3-AS1 to inhibit cell proliferation. (A). CCK-8 assay was performed to analyze the roles of miR-143-3p and PSMG3-AS1 in regulating the proliferation of SNU-182 and SNU-398 cells. The cell proliferation rate in the PSMG3-AS1 group increased significantly, while the miR-143-3p group decreased. There was no significant difference in cell proliferation between the PSMG3-AS1 and miR-143-3p co-transfection groups compared with the group C. B and C are colony formation experiments in SNU-182 (B) and SNU-398 (C). The colony formation of miR-143-3p group was significantly lower than that of group C, while the PSMG3-AS1 group was higher. The number of colonies formed in PSMG3-AS1 and miR-143-3p groups was not significantly different from that in group C. All experiments were performed in 3 replicates and mean values were presented and compared. *p < 0.05.
Conclusion
In conclusion, PSMG-AS1 is overexpressed in HCC. miR-142-3p can target PSMG3-AS1 to suppress cancer cell proliferation.

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Disclosure
The authors declare that they have no conflicts of interest.

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