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Regular paper

MicroRNA-508-3p regulates the proliferation of human lung cancer cells by targeting G1 to S phase transition 1 (GSPT1) protein

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Purpose: Due to its crucial cancer regulatory role, microRNA-508-3p has been reported as a potential therapeutic anticancer molecular target. The present work encompassed the molecular characterization of microRNA-508-3p in lung cancer emphasizing on understanding the possible mechanism of its regulatory action. Methods: qRT-PCR was performed to estimate the relative gene expression of microRNA-508-p in the tissue samples. The proliferation of cancer cells was determined by cell counting kit-8. The colony formation from cancer cells was analyzed by clonogenic assay. Mitotic phase distribution was understood by employing the flow cytometric technique. Edu-Hoechst staining was used for the assessment of cell viability. *In silico* analysis and dual-luciferase assay were used for target identification of microRNA-508-3p in lung cancer. Immunofluorescence and western blotting studies were carried out for relative protein expression. The rat models were used for performing the *in vivo* experimental procedures. Results: The study showed the significant down-regulation of microRNA-508-3p in lung cancer. The lower expression levels of microRNA-508-3p were shown to be associated with poor survival of lung cancer patients. The over-expression of microRNA-508-3p was found to decline the proliferation and viability of cancer cells together with the induction of mitotic cell cycle arrest at G1 by targeting G1 to S phase transition 1 (GSPT1) protein. MicroRNA-508-3p up-regulation inhibited the in vivo tumor growth in rat models. Conclusion: Our study identifies miR-508-3p as a pivotal regulator of lung cancer cell proliferation by targeting the GSPT1 protein. This highlights its potential as a tumor suppressor and a therapeutic target for lung cancer. Our findings offer mechanistic insights into miRNA-mediated cancer progression, prompting further research in this intricate regulatory network.

Keywords: Lung cancer, micro-RNA, proliferation, qRT-PCR, immunefluorescence staining, cell cycle arrest, G1 to S phase transition 1

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Abbreviations: 3'UTR, 3'-Untranslated region; eRF, 1Eukaryotic release factor 1; GSPT1, G1 to S phase transition 1; miRNA, MicroRNA; mRNA, Messenger RNA; qRT-PCR, Quantitative Real Time PCR; SCC, Esophageal squamous cell carcinoma

INTRODUCTION

Lung cancer is the most commonly diagnosed human cancer worldwide and accounts for the highest mortality rate among both sexes of the human population at the global level (Thandra *et al.*, 2021; Malhotra *et al.*, 2016). On average, about 1.2 million new lung cancer cases are reported annually, worldwide (Cheng *et al.*, 2016). In 2018, more than 2 million lung cancer cases were diagnosed and the total number of deaths from lung cancer was 1.76 million (Bray *et al.*, 2018). Lung cancer has a very poor prognosis and the 5-year survival rates range between 10–20% making it a very destructive disorder (Hirsch *et al.*, 2017). In recent times, scientists have suggested an in-depth understanding of the molecular mechanics of lung cancer primarily aiming at the exploration of various genetic alterations to develop better prognostic and therapeutic measures against the severity of this dominant malignancy (Rong & Yang, 2018). In this regard, the research investigations apart from focusing on the protein-coding genes have laid considerable stress on the characterization of non-coding RNAs including micro-RNAs (miRNAs) for their cancer regulatory role (Bhan *et al.*, 2017).

The miRNAs include the class of very short, single-stranded and non-coding RNAs which typically regulate the gene expression in eukaryotes at post-transcriptional/translational level through mRNA degradation or by restraining the translation of eukaryotic genes through direct interaction with their 3'-untranslated region (3'-UTR; O'Brien *et al.*, 2018). The miRNAs have been reported to regulate the vital aspects of human physiology and have an important role in disease development. The miRNAs exhibit altered expression in many human cancers suggesting their possible role in cancer growth and proliferation (D'Angelo *et al.*, 2016). Several miRNAs were found to be dysregulated in lung cancer and these were proposed to act as prognostic biomarkers against lung cancer (Wu *et al.*, 2019). MicroRNA-508-3p (miRNA-508-3p) has been shown to regulate the tumor growth and proliferation of many human cancers like gastric and liver cancers (Shang *et al.*, 2016; Wu *et al.*, 2017). miR-508-3p exhibits reduced expression in cancer cells displaying resistance to treatment, which correlates with unfavorable survival outcomes (Zhao *et al.*, 2019). Additionally, diminished levels of miR-508-3p in breast cancer are linked to the occurrence of distant and lymph node metastases. Furthermore, within the same cluster of miRNAs, including miR-508-3p, miR-509-3p, miR-509-3-5p, and miR-514a-3p, there is a frequent downregulation in advanced and recurrent ovarian carcinoma (Bagnoli *et al.*, 2011), implying their coordinated regulation and co-expression. Various studies have also highlighted the prognostic relevance of these miRNAs in diverse cancer types (Hiramoto *et al.*, 2017; Shang *et al.*, 2014), although

exceptions exist. For instance, in esophageal squamous cell carcinoma (SCC), elevated miR-508-3p expression is paradoxically associated with shorter disease-free and overall survival. The growth regulatory role of microRNA-508-3p in lung cancer tumorigenesis has not been worked out.

The G1 to S phase transition 1 (GSPT1) protein is pivotal in orchestrating cell cycle progression, making it a significant player in cancer biology (Dang *et al*., 2017). Its involvement in cancer pathogenesis has garnered attention across various cancer types, with an emerging focus on its implications within lung cancer. GSPT1's essential role lies in mediating the transition of cells from the growth-arrested G1 phase to the proliferative S phase of the cell cycle (Nishiguchi *et al*., 2021). By regulating the translation termination process, GSPT1 controls gene expression, impacting fundamental cellular functions such as DNA replication and cell division. Its role extends beyond its involvement in the cell cycle, as GSPT1 has been implicated in cellular stress responses, mRNA quality control, and RNA metabolism.

In the context of cancer, GSPT1's dysregulation contributes to tumorigenesis through various mechanisms. Elevated GSPT1 expression has been associated with increased proliferation rates, reduced apoptosis, and altered gene expression patterns, all hallmark cancer cell features (Long *et al*., 2021). Additionally, GSPT1's potential involvement in angiogenesis and metastasis underscores its multifaceted contributions to cancer progression (Tian *et al*., 2018). Specifically, in lung cancer, GSPT1's perturbation has garnered attention due to its potential role in disease pathogenesis. Altered GSPT1 expression has been reported in lung cancer tissues, and its overexpression has been correlated with unfavorable clinical outcomes (Cheng *et al*., 2021). GSPT1's regulatory influence over cellular processes in lung cancer aligns with its broader roles in other cancer types.

Understanding GSPT1's intricate role in cancer development and progression opens up avenues for therapeutic exploration. Targeting GSPT1 holds promise as a strategy to curtail uncontrolled proliferation and enhance the efficacy of cancer treatments. The intricate interplay of GSPT1 within cellular pathways underscores its potential as a druggable target. The primary objective of this study is to delve into the pivotal role played by miRNA-508-3p in governing the proliferation of human lung cancer cells. This investigation centers on the specific mechanism through which miRNA-508-3p exerts its influence, by targeting the GSPT1.

MATERIALS AND METHODS

Human clinical samples and cell lines

Sixty-five (65) paired specimens of lung cancer and normal adjacent tissues (taken from tumor margins and representing pathologically normal lung tissues) were collected from lung cancer patients after surgery at The Affiliated Hospital of Nantong University from 2014 to 2018. The patients didn't receive chemo or radiotherapy during tissue collection. The patients were informed about the study in advance and tissues were collected only after consent. The tissues were frozen immediately after collection in liquid nitrogen and then stored at -80°C till experimental use. The study was approved by the institutional ethical guidelines committee. The study has been approved by the ethics committee of The Affiliated Hospital of Nantong University (No: AADEX124).

Four lung cancer cell lines (A427, A549, SK-LU-1 and HCC827) and the normal lung cell line (MRC5) were purchased from the American Type Culture Collection and their propagation was performed at 37°C with 5% CO_2. Dulbecco's Modified Eagle's Medium (DMEM, Gibco BRL) containing 10% FBS (v/v, Hyclone) was used as the cell culturing medium.

For transfection purposes, lung cancer cell lines (2×10^5 per well) were seeded in 6-well plates and allowed to attach for at least 18 h. miR-508 mimics (100 pmol), si-GSPT1 (100 pmol) together with their negative controls (100 pmol; designed and synthesized by RiboBio, Guangzhou, China) were transfected into cells using Lipofectamine 2000 reagent (Thermo Fisher Scientific) according to manufacturer guidelines. Over-expression of GSPT1 was achieved using the over-expression vector pcDNA3.1 (4 μg). The transfections were performed at 37°C for 48 h. Total RNA and protein were collected 48 hours post-transfection.

RNA isolation and qRT-PCR

Total cellular RNA from tissues and cell lines was extracted using the Trizol method (Thermo Fisher Scientific) which was reverse transcribed to cDNA with Reverse Transcription Kit (Takara). The following thermal cycling conditions were applied: 16°C for 30 min, 42°C for 30 min and 85°C for 5 min. The gene expression analysis was performed through qRT-PCR with the help of SYBR Green PCR Master Mix (Thermo Fisher Scientific). The qRT-PCR procedure was executed according to the following temperature protocol: an initial step at 50°C for 2 minutes, followed by denaturation at 95°C for 10 minutes. Subsequently, 40 cycles were performed, involving denaturation at 95°C for 15 seconds, followed by an annealing/extension step at 60°C for 60 seconds. Relative expression levels were estimated by the $2^{-\Delta\Delta CT}$ method. U6 was used as the internal expression control. The primer sequences used were as follows: miR-508-3p forward, 5'-TTCAAGAGACATGAGTGAC-3' and reverse, 5'-TCTCTTGAACATGAGTGACG-3'; U6 forward, 5'-TGCGGGTGCTCGCTTCGGCAGC-3' and reverse, 5'-CCAGTGCAGGGTCCGAGGT-3.

Proliferation and clonogenic assays

The proliferation of stably transfected lung cancer cells was analyzed through an MTT assay. The transfected cells were incubated in 96-well plates at 37°C for 0 h, 20 h, 40 h, 60 h, 80 h or 100 h prior to the addition of MTT reagent (0.5% final concentration) to wells. The plate for incubated for 4 h again after adding MTT. Then the culture medium was replaced with 250 μl DMSO to dissolve the formazan crystals. Using a microplate reader (ELx808™, Agilent, USA), the optical density (OD) values were recorded at 570 nm for each well. These values were used for analyzing the relative cell proliferation using the formula: Cell viability (%) = (Absorbance of treated samples/Absorbance of control samples) ×100.

After their stable transfection for 48 hours, the cells at a cellular density of 500 cells per well were propagated for 14 days at 37°C. The colonies were then fixed with methanol for 20 min and stained with 1.0% crystal violet. The relative colony number was estimated by manually counting the colonies in 10 random fields under a microscope.

Cell cycle analysis

The stably transfected cancer cells were cultured in the 12-well plates at 37°C for 48 h using an initial cellular concentration of 1.6×10^6 cells/well. The cells were harvested and fixed with 70% ethanol at 4°C overnight. Staining of cells with 50 µg/ml propidium iodide (Thermo Fisher Scientific) followed by flow cytometric analysis was carried out for studying the cancer cell mitosis. The fluorescence-activated cell sorting (FACS) Calibur and Cell Quest software were used for estimating the cell cycle phase distribution of cancer cells.

Edu-Hoechst staining

Approximately 0.5×10^5 transfected cells were seeded per well of the 96-well plate. Each well was added to the EdU-medium for 2.5 h. The cells were then washed with PBS and ethanol fixed. The EdU assay kit (RiboBio) was used for viability assessments. The cells were also incubated with Hoechst solution for whole sample staining.

In vivo experiments

The nude rat xenografts were obtained using five- to six-week-old BALB/c nude male rats. The rats were distributed into 2 groups (10 rats per group). The rats were maintained using the institute's central animal house facility. The animal flanks were subcutaneously injected with 5×10^6 matrigel suspended A549 cancer cells to obtain the xenograft models. After tumor induction, the rats were treated with intra-tumor injections carrying miR-508-3p mimics or its negative control on alternate days. The intra-tumor injections were given for a varied number of days. On the 25th day after tumor induction, the rats were sacrificed and rat tumors were rescued. The size and weight of tumors were determined and used for calculating the tumor volume. The protein levels of PCNA and Cyclin D1 in rat tumors were analyzed through immune-histochemical staining.

Western blotting

Total cellular proteins were extracted by treating the cells with RIPA lysis and extraction buffer (Thermo Fisher Scientific). The protein expression was determined through chemiluminescence after PAGE and protein blotting using specifically designed primary antibodies like anti-GSPT1 (Novus Biologicals, NBP2-16754, 1;1000) and anti-actin (Sigma Aldrich, A2066, 1:1000). After overnight incubation, followed by proper washing, blots were incubated with HRP-linked secondary antibody (Cell Signaling Technology, 7074, 1:2000). Human actin protein served as the internal reference in western blotting study.

Bioinformatics and dual luciferase assay

To predict the molecular targets of miRNA-508, online miRNA target prediction software like Starbase (http://starbase.sysu.edu.cn) and TargetScan (http://www.targetscan.org/) were used. The latter also predicted the microRNA-508-3p binding site in the 3'-UTR of GSPT1. The prediction was assessed by luciferase assay using a dual luciferase reporter assay system (Promega). The 3'-UTR binding site (in native, WT or mutated, MUT state) bearing stretch of GSPT1 was cloned into the pGL3 vector for generating the reporter construct. The latter was co-transfected with miR-508-3p mimics or miR-NC into A549 cancer cells. The luciferase activity measurements were performed as per the manufacturer's protocol.

Statistical analysis

The experiments carried three replicates and the final data represented mean± S.D. Student's t-test and one-way ANOVA were performed using SPSS software (SPSS 22.0, Chicago, IL, USA) to analyze the statistical difference between the two values performed. The *p*-values <0.05 were deemed to represent a statistically significant difference between the two values.

RESULTS

Decreased microRNA-508-3p correlates with poor survival of lung cancer patients

The gene expression of microRNA-508-3p was determined from 65 of each lung cancer tissue samples and normal adjacent tissues through qRT-PCR. It was found that microRNA-508-3p has significantly lower expression in lung cancer tissues in comparison to normal lung tissues (Fig. 1A). The analysis of microRNA-508-3p expression from the paired cancerous and normal adjacent samples also revealed a similar inference (Fig. 1B). Interestingly, the 5-year study on the survival period of the patients from the month of detection of lung cancer receiving the similar therapy procedures was analyzed with the expression level of miRNA-508. It was found that the expression of microRNA-508-3p positively correlated with the period of patient's survival (months), i.e., the higher the expression of miRNA-508, the longer the survival period (Fig. 1C). Further, when the expression of miR-508 was determined in four lung cancer cell lines (A427, A549, SK-LU-1 and HCC827) and compared with its expression in a normal lung cell line (MRC5);

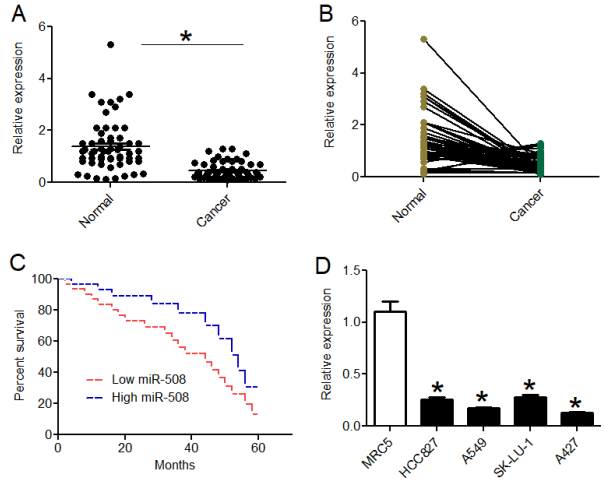

Figure 1. Decreased microRNA-508-3p correlates with poor survival of lung cancer patients.
(**A**) Lower microRNA-508-3p expression in lung cancer tissues in comparison to the normal lung tissues. (**B**) microRNA-508-3p expression from the paired cancerous and normal adjacent samples revealed a similar inference. (**C**) microRNA-508-3p expression positively correlated with the period of patient's survival (months), i.e., the higher the expression of miRNA-508, the higher the survival period. (**D**) qRT-PCR expression analysis revealing significant down regulation of microRNA-508-3p in lung cancer cell lines (A427, A549, SK-LU-1 and HCC827) in comparison to normal lung cell line (MRC5). Experiments were carried out in three replicates. (*) represents statistical significance at $p<0.05$ *vs* normal control.

it was seen that the panel of lung cancer cell lines exhibited significantly lower microRNA-508-3p expression in comparison to the normal lung cell line (Fig. 1D). The cancer cell lines, A427 and A549 were seen to exhibit the least expression among the four cancer cell lines used and were thus used for further characterization of miRNA-508. The results thus reveal significant repression of microRNA-508-3p in lung cancer and its decreased expression negatively correlating with lung cancer patient survival.

MicroRNA-508-3p overexpression declined the cancer cell growth and colony formation

To infer whether the lowered expression level of microRNA-508-3p has any doing with the lung cancer cell growth, microRNA-508-3p was over-expressed in A549 and A427 cancer cell lines and its over-expression was confirmed by RT-PCR (Fig. 2A). Both the cell lines over-expressing microRNA-508-3p showed significantly lower proliferation in comparison to the respective negative control cells (Fig. 2B). Again, the colony formation was markedly reduced by microRNA-508-3p over-expression (Fig. 2C). Both A549 and A427 cancer cell lines exhibited almost 50 % lower colony formation when microRNA-508-3p was over-expressed in them (Fig. 2D). The results suggest that microRNA-508-3p negatively regulates the growth of lung cancer cells and higher proliferation of cancer cells might be achieved through lowered microRNA-508-3p down-regulation among other regulatory factors.

The cell cycle was arrested at the G1 phase in cancer cells over-expressing miRNA-508

The flow cytometric analysis of A427 and A549 cancer cells over-expressing microRNA-508-3p was performed to study the mitotic phase distribution which was compared with the negative control cells. A significantly higher percentage of cancer cells was shown to depict the Go/G1 cell cycle phase for the cancer cells over-expressing microRNA-508-3p while the percentage of cells was significantly lower exhibiting S and G2/M when compared with the phase distribution of negative control cells (Fig. 3A). To further validate the finding, the cancer cells over-expressing microRNA-508-3p in comparison with negative control cells were stained with EdU fluorescent stain to detect the relative number of cells which had undergone DNA synthesis. The number of EdU-stained cells was significantly lower under microRNA-508-3p overexpression in comparison to negative control cells (Fig. 3B). The percent number of EdU-positive cells was as low as 10% for both A549 and A427 cancer cell lines over-expressing microRNA-508-3p when compared with the respective negative control cells (Fig. 3C). Together, the results indicated that microRNA-508-3p overexpression induced lung cancer cells with cell cycle arrest at the G1 stage and thus reduced their *in vitro* proliferation.

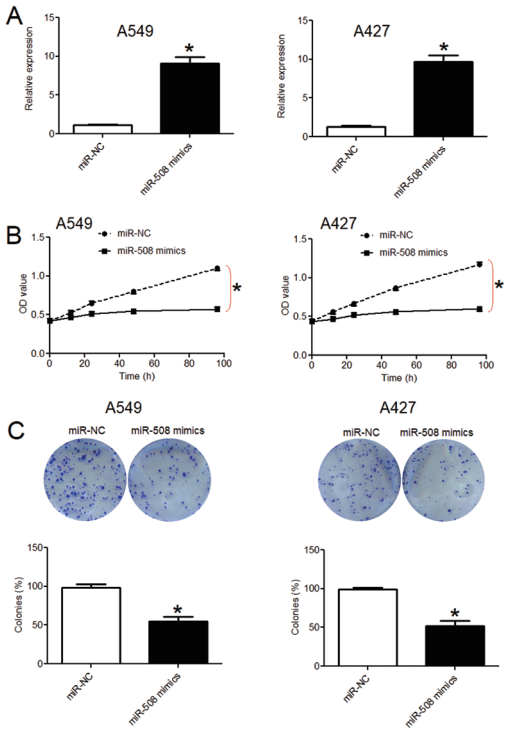

Figure 2. MicroRNA-508-3p over-expression declined the cancer cell growth and colony formation.
(**A**) A549 and A427 cells transfected with microRNA-508-3p mimics showing significant up-regulation of microRNA-508-3p in comparison to respective miR-NC transfected cells. (**B**) A549 and A427 cells overexpressing microRNA-508-3p exhibit limited growth *in vitro* than the respective miR-NC transfected cells. (**C**) A549 and A427 cells over expressing microRNA-508-3p exhibit significantly lower colony formation than the respective miR-NC transfected cells (magnification ×100). Experiments were carried out in three replicates. (*) represents statistical significance at $p<0.05$ *vs* miR-NC.

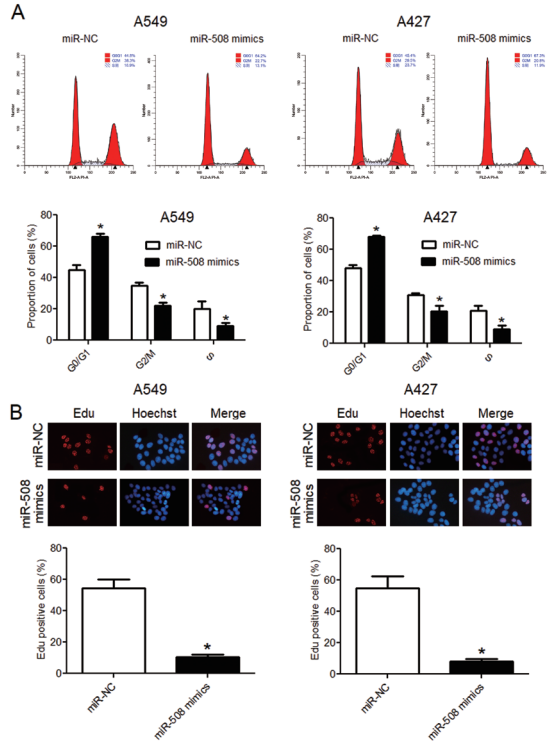

Figure 3. Cell cycle was arrested at the G1 phase in cancer cells overexpressing miRNA-508.
(**A**) Flow cytometry analysis of A549 and A427 cells showed that overexpression of microRNA-508-3p produces a significantly higher percentage of cancer cells in the Go/G1 cell cycle phase while s significantly lower percentage of cancer cells in S and G2/M compared with the phase distribution of negative control cells. (**B**) A549 and A427 cells overexpressing microRNA-508-3p showed a lower number of EdU stained cells (10%) than the respective miR-NC transfected cells (magnification ×200). Experiments were carried out in three replicates. (*) represents statistical significance at $p<0.05$ *vs* miR-NC.

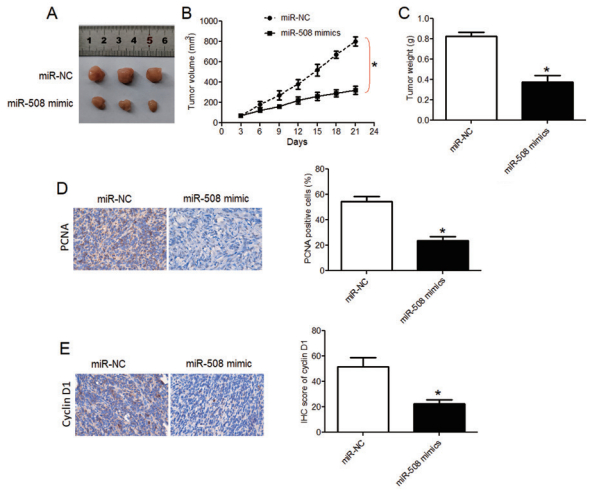

Figure 4. MicroRNA-508-3p up-regulation inhibited in vivo rat tumorigenesis.
(**A**) Morphological comparison of the rat tumors rescued from the animals overexpressing miR-NC and microRNA-508-3p mimic. (**B**) *In vivo* rat tumor volume was significantly decreased under microRNA-508-3p over-expression than the respective miR-NC. (**C**) Tumor weight was significantly decreased under microRNA-508-3p over-expression than the respective miR-NC. (**D, E**) The IHC staining of PCNA revealed that tumors exhibited significantly lower PCNA protein expression under microRNA-508-3p over-expression and a similar observation was made about the expression level of cyclin D1 (magnification ×20). Experiments were carried out in three replicates. (*) represents statistical significance at $p<0.05$ *vs* miR-NC.

MicroRNA-508-3p up-regulation inhibited *in vivo* rat tumorogenesis

During the *in vivo* study, the intra-tumor overexpression was achieved in the rat models. The morphological comparison of the rat tumors rescued from the animals after their sacrifice revealed that the rat tumor size was significantly lower under microRNA-508-3p over-expression when compared with that of the negative control rats (Fig. 4A). Similarly, the *in vivo* rat tumor volume was significantly decreased under microRNA-508-3p over-expression (Fig. 4B). Tumor weight also showed a similar trend (Fig. 4C). The immune-histochemical staining of proliferating cell nuclear antigen (PCNA) revealed that rat tumors exhibited significantly lower PCNA protein expression under microRNA-508-3p overexpression and a similar observation was made about the expression level of cyclin D1, both of which are used are the proliferation markers (Fig. 4D and 4E). Hence, the results suggest that up-regulation of microRNA-508-3p inhibited the growth and proliferation of *in vivo* rat tumors indicating its tumor-suppressive role in lung cancer.

MicroRNA-508-3p exercised its effects by targeting GSPT1 in lung cancer

To look for the possible regulatory mechanism of action of microRNA-508-3p in lung cancer, online bioinformatics was performed. MicroRNA-508-3p was predicted to be targeting G1 to S phase transition 1 (GSPT1) protein (Fig. 5A). MicroRNA-508-3p was shown to specifically bind with the 3'-UTR of GSPT1 in sequence-specific fashion (Fig. 5B). The dual luciferase reporter assay was performed to confirm this prediction. The significant reduction in the luciferase activity when the overexpression construct of microRNA-508-3p was co-transfected with the native (WT) reporter construct

A

Target gene	Representative transcript	Gene name	Number of 3P-seq tags supporting UTR + 5	Link to sites in UTRs	Site counts Total	Representative miRNA	Total context ++ score
GSPT1	ENST00000434724.2	G1 to S phase transition 1	1119	Sites in UTRs	5	Has-miR-508-3p	-0.95

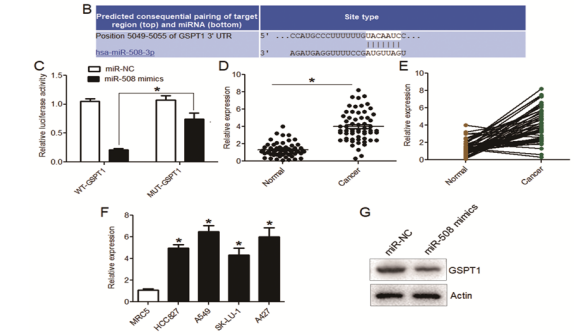

Figure 5. MicroRNA-508-3p exercised its effects by targeting GSPT1 in lung cancer.
(**A**) TargetScan software predicted that microRNA-508-3p targets the GSPT1 protein. (**B**) *In silico* analysis predicted a specific microRNA-508-3p binding site in 3'-UTR of GSPT1. (**C**) The significant reduction in the luciferase activity when the overexpression construct of microRNA-508-3p was co-transfected with the native (WT) reporter construct of GSPT1 in A549 cancer cells showed that microRNA-508-3p interacted with 3'-UTR of GSPT1. (**D**) Expression of GSPT1 in normal and lung cancer tissues. (**E**) GSPT1 expression from the paired cancerous and normal adjacent samples revealed a similar inference. (**F**) Expression analysis of GSPT1 in lung cancer cell lines in comparison to normal liver epithelial cell line. (**G**) Over-expression of microRNA-508-3p in A549 cancer cells negatively affected the protein level of GSPT1 suggesting post-transcriptional/translational targeting of the latter by microRNA-508-3p in lung cancer. Experiments were carried out in three replicates. (*) represents statistical significance at $p<0.05$ *vs* miR-NC.

of GSPT1 in A549 cancer cells showed that microRNA-508-3p interacted with 3'-UTR of GSPT1 (Fig. 5C). Further support was attained from the expression study of the GSPT1 gene from lung cancer tissues and cancer cell lines in comparison to normal tissues and cell line. GSPT1 was found to have significantly higher expression in cancer tissues and cell lines corresponding to lower microRNA-508-3p expression (Fig. 5D, 5E and 5F). Further, the over-expression of microRNA-508-3p in A549 cancer cells negatively affected the protein level of GSPT1 suggesting post-transcriptional/translational targeting of the latter by microRNA-508-3p in lung cancer (Fig. 5G).

MicroRNA-508-3p directly binds to GSPT1 in lung cancer

To explore whether the observed effects of microRNA-508-3p against lung cancer were modulated through GSPT1 targeting, RNA interference-mediated silencing of GSPT1 was performed in A549 cancer cells and repression was confirmed by qRT-PCR (Fig. 6A). The cancer cells exhibited significantly lower proliferation under GSPT1 silencing (Fig. 6B). GSPT1 silencing was seen to induce Go/G1 phase cell cycle arrest (Fig. 6C). Similar to microRNA-508-3p over-expression, the down-regulation of GSPT1 reduced the proliferative viability of lung cancer cells (Fig. 6D). Confirming the execution of microRNA-508-3p role in lung cancer through GSPT1, the over-expression of GSPT1 in A549 cancer cells minimized the antiproliferative effects of microRNA-508-3p up-regulation (Fig. 6E). Summing up, the results are suggestive of direct binding of microRNA-508-3p with GSPT1 to down-regulate the expression of latter translationally in lung cancer and exertion of mi-

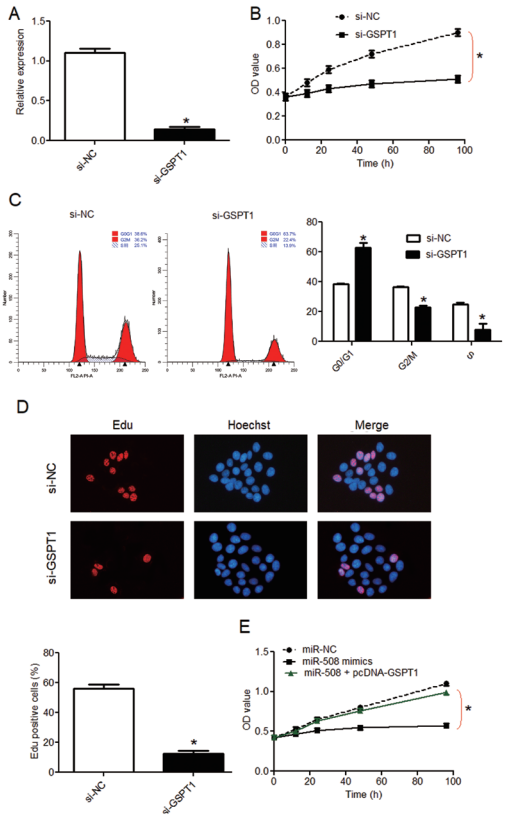

Figure 6. MicroRNA-508-3p directly binds to GSPT1 in lung cancer.
(**A**) Relative expression of GSPT1 in A549 cancer cells after silencing of GSPT compared to si-NC. (**B**) A549 cancer cells exhibited significantly lower proliferation under GSPT1 silencing compared to cells transfected with si-NC. (**C**) A549 cancer cells with GSPT1 silencing were seen to induce Go/G1 phase cell cycle arrest compared to cells transfected with si-NC. (**D**) A549 cancer cells transfected with si-GSPT1 were found to reduce the proliferative viability of lung cancer cells compared to cells transfected with si-NC. (**E**) Over-expression of GSPT1 in A549 cancer cells minimized the anti-proliferative effects of microRNA-508-3p up-regulation compared to cells tranfected with si-NC. Experiments were carried out in three replicates. (*) represents statistical significance at $p<0.05$ *vs* si-NC.

croRNA-508-3p tumorogenesis regulatory role through miRNA-508/GSPT1 molecular axis which further insights into the therapeutic value of miRNA-508/GSPT1 axis against the deadliest cancer, the human lung cancer.

DISCUSSION

Lung cancer is by far the deadliest human cancer with high incidence and mortality rates worldwide (Busch *et al.*, 2016). The disease is reportedly induced through genetic alterations and external environmental factors (Samet, 2013). A thorough understanding of lung cancer progression is essential for formulating effective treatment measures against this lethal menace. At the molecular level, the studies have focused on exploring different regulatory molecules regulating the growth and propagation of lung cancer including the non-coding RNAs (Lin & Yang, 2018). Falling in the category of non-coding RNAs, the micro-RNAs (miRNAs) were shown to profoundly affect the tumorogenesis of many human cancers (Vannini *et al.*, 2018). Many miRNAs have been shown to exercise a key developmental role in human lung cancer (Kabekkodu *et al.*, 2020). Although microRNA-508-3p has been found to exhibit active involvement in a number of cancer types, but its in-depth role has not been explored in lung cancer. The current study revealed a significant transcriptional down-regulation of microRNA-508-3p in lung cancer. microRNA-508-3p has also been elucidated to exhibit transcriptional repression in human cancers like gastric cancer, and colon cancer (Wang & Jin, 2019). Additionally, the level of microRNA-508-3p down-regulation negatively correlated with the survival of lung cancer patients. This indicated that microRNA-508-3p functions as a tumor suppressor in lung cancer and other human cancers, as reported by earlier studies (Huang *et al.*, 2016). The microRNA-508-3p over-expression declined lung cancer growth both *in vitro* and *in vivo*. The anti-cancer effects of microRNA-508-3p up-regulation were also evidenced as the arrest of lung cancer cell cycle at the G1 subphase of mitotic interphase, which suggests that microRNA-508-3p negatively regulates lung cancer cell growth as has been supported by previous studies (Bao *et al.*, 2016). Importantly, the current study showed that G1 to S phase transition 1 (GSPT1) protein is functionally targeted by microRNA-508-3p in lung cancer. The role of microRNA-508-3p was found to be attenuated via GSPT1. The GSPT1 is a protein-coding gene that regulates the translational termination in eukaryotes associated with eukaryotic release factor 1 (eRF1; Zhouravleva *et al.*, 2006). It is involved in cell cycle regulation and cell apoptosis. In the present study, the GSPT1 over-expression resulted from microRNA-508-3p repression and the former was proved to considerably affect the molecular level progression of lung cancer. The up-regulation of GSPT1 was also reported to be linked with the growth and development of gastric and breast cancer (Tian *et al.*, 2018). In another report, the expression of GSPT1 was shown to be significantly higher in non-small lung cancer cells and negatively correlated with miRNA-27b-3p expression (Sun *et al.*, 2019). The miRNA/GSPT1 axes have been suggested to act as potential therapeutic targets against human cancers including lung cancer (Li *et al.*, 2020). Concluding with a similar inference, the miRNA-508/GSPT1 was unravelled as a vital growth regulatory link in lung cancer. The results of the present study support its therapeutic utility against human lung cancer.

The functional analyses conducted in this study provide mechanistic insights into how microRNA-508-3p exerts its tumor-suppressive effects in lung cancer. The inhibition of cancer cell proliferation and viability following microRNA-508-3p overexpression aligns with the notion that this microRNA acts as a negative regulator of tumor growth. The induction of cell cycle arrest at the G1 phase, as evidenced by the down-regulation of the GSPT1 protein, suggests that microRNA-508-3p exerts its antiproliferative effects by interfering with key cell cycle regulatory pathways. These findings offer a clearer picture of the molecular events modulated by microRNA-508-3p that contribute to its tumor-suppressive functions.

The in vivo experiments using rat models provide a relevant and translational dimension to the study. The observed inhibition of tumor growth in response to microRNA-508-3p up-regulation underscores the potential therapeutic application of this microRNA in lung cancer treatment. These results add to the growing body of evidence supporting microRNA-based therapies as promising avenues for cancer intervention.

It is worth noting that the findings of this study resonate with the broader understanding of microRNA dysregulation in cancer and reinforce the idea that targeting

specific microRNAs could hold immense therapeutic potential. However, further investigations are warranted to delve deeper into the exact mechanisms through which miRNA-508-3p modulates its target gene(s), potentially involving intricate regulatory networks that influence cancer progression.

The current study significantly contributes to our understanding of the role of microRNA-508-3p in lung cancer. The comprehensive molecular characterization, functional analyses, and in vivo experiments underscore the potential of microRNA-508-3p as a promising therapeutic target for lung cancer treatment. By elucidating its regulatory mechanisms and demonstrating its impact on tumor growth, this research opens up avenues for developing innovative therapeutic strategies to improve patient outcomes in lung cancer management.

CONCLUSIONS

The current study showed that microRNA-508-3p experiences a key regulatory role in the growth and proliferation of lung cancer, and the lowered expression levels of microRNA-508-3p account for poor disease survival. The results were conclusive of the prognostic and therapeutic role of microRNA-508-3p against lung cancer, which might be utilized as a novel and alternative anticancer therapy for the treatment of lung cancer.

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