**Original Article**

**Increased expression of IncBRM predicts poor prognosis in lung cancer and regulates cell proliferation and apoptosis through Sox4**

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**Abstract:** Lung cancer is the most frequent and deadliest cancer, worldwide, especially in China. However, the molecular mechanisms involved in lung cancer development remain unclear. Moreover, IncBRM (long non-coding RNA for association with Brahma [BRM]; gene symbol LINCR-0003), which is highly transcribed in liver cancer stem cells, has been associated with progression and prognosis of hepatocellular carcinoma. However, the roles of IncBRM in lung cancer have not been thoroughly examined. The current study investigated the roles of IncBRM in lung tumorigenesis. It was found that expression of IncBRM was highly increased in samples obtained from patients with lung cancer, compared with healthy samples, indicating poor prognosis. In addition, data from quantitative real-time polymerase chain reaction analysis showed that expression of IncBRM differed between several lung cancer cell lines, with the highest levels observed in A549 cells. Moreover, IncBRM knockdown inhibited the proliferation of lung cancer cells by affecting the cell cycle in vitro, suppressing tumor growth in a xenograft tumor model. Furthermore, IncBRM knockdown promoted the apoptosis of lung cancer cell lines. Mechanistically, expression of Sox4, a key factor in the development of lung cancer, was compromised after IncBRM knockdown. Moreover, results suggest that IncBRM competitively interacts with miR-211-5p and regulates Sox4 expression. Collectively, present findings reveal, for the first time, the role of IncBRM as a tumor promoter in lung cancer. Thus, IncBRM is a potential prognostic biomarker and therapeutic target for lung cancer.

**Keywords:** Lung cancer, IncBRM, Sox4, proliferation, apoptosis

**Introduction**

Lung cancer is the most common type of cancer, worldwide, and the leading cause of cancer-related deaths in China. This may be due to the limited therapeutic options in advanced stages of the disease [1, 2]. Although advancements have been made in the clinical treatment of lung cancer in recent years, the overall survival (OS) time of patients has not improved significantly. An important reason for the lack of improvement is the limited understanding of lung cancer pathogenesis. Thus, a more comprehensive understanding of the molecular mechanisms underlying carcinogenesis in the lungs and identification of new therapeutic targets will undoubtedly improve outcomes in the future.

Using next-generation sequencing technologies, it has been determined that long non-coding RNAs (lncRNAs) are widely transcribed in the genome [3, 4]. Moreover, lncRNAs are transcripts longer than 200 nucleotides without protein-coding potential [5]. Although previously characterized as transcriptional “noise”, emerging evidence has shown that lncRNAs play critical roles in various biological processes, including cellular development and differentiation [6-8]. Furthermore, abnormal expression of lncRNAs has been linked to the development and progression of cancer, leading to dysregulation of cancer cell proliferation, apoptosis, migration, and invasion [9-11]. For example, the oncogenic IncRNA HOX Transcript Antisense Intergenic RNA (HOTAIR) may be used to predict prognosis in patients with non-small-cell lung cancer, determining the usefulness of chemotherapy in patients [12, 13]. Therefore, identification of key oncogenic lung cancer-related lncRNAs is necessary in developing improved strategies for lung cancer treatment.
Additionally, lncRNA for association with Brahma (lncBRM), located on chromosome 5 between the PLK2 and ACTBL2 genes, is highly expressed in liver cancer stem cells and hepatocellular carcinoma tumors. Subsequently, highly expressed lncBRM activates YAP1 signaling to promote self-renewal of liver cancer stem cells, initiating tumor expansion [14]. However, evidence regarding the function of lncBRM in lung cancer tumorigenesis and progression has been limited. The aim of the present study was to investigate the biological roles and molecular mechanisms of lncBRM in lung cancer. The current study determined expression patterns of lncBRM in lung cancer tissues and matched adjacent non-tumor tissues, examining the correlation with prognosis. In addition, this study explored the function of lncBRM using in vitro and in vivo assays. Finally, the current study identified Sox4 as a target of lncBRM, possibly affecting the proliferation of lung cancer cells.

Materials and methods

Clinical samples

A total of 70 fresh lung cancer tissue samples and matched adjacent non-tumor tissues were obtained from patients that underwent surgical resections at Shenzhen Luohu People’s Hospital, between June 2015 and May 2016. All specimens were immediately frozen and stored in liquid nitrogen until RNA extraction. The tumor samples were pathologically confirmed by pathologists. The current study was approved by the Ethics Committee of Shenzhen University and performed in compliance with the principles of the Declaration of Helsinki. All patients provided informed consent prior to participation. Patients discharged from the hospital were followed-up routinely, about every 3 months, according to a scheduled program. Clinicopathological characteristics of the patients with lung cancer are summarized in Table 1.

**Table 1. Clinicopathological factors of lung cancer patients**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Expression of lncBRM</th>
<th>P-value</th>
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Healthy lung epithelial cell lines (MRC-5) and lung cancer cell lines (non-small-cell lung cancer cells A549, NCL-H1650, NCI-H1299, HCC-827, SPC-A1, and MSTO-211H) were obtained from the Cell Bank of Chinese Academy of Science (Shanghai, China). All cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) (GIBCO-BRL, Grand Island, NY, USA), with 10% fetal bovine serum (Invitrogen, Grand Island, NY, USA). All cells were incubated at 37°C in 5% CO₂ humidified air.

Small interfering RNA (siRNA) transfection and Sox4 transfection

This study used siRNA for knockdown of lncBRM in lung cancer cell lines. Sequences of the oligonucleotides were as follows: Control siRNA, 5’-GGAATATTGTCGACTTAAC-3’; lncBRM siRNA, 5’-GGACCACTAGGTTTCATAT-3’.

For transfection, the cells were seeded in 12-well plates at a density of 5 × 10⁴ cells/well. After 24 hours and 70-80% confluence, the cells were transfected with respective lncBRM siRNAs (50 nM) and control-siRNAs (50 nM) in serum-free medium using Lipofectamine 2000™ (Invitrogen), according to manufacturer instructions. After incubation for 6 hours at 37°C, the medium in each well was replaced with DMEM containing 10% heat-inactivated fetal bovine serum. The protocol for transfection of Sox4 was identical to the abovementioned protocol, except for siRNA (replaced with the pCDNA-Sox4 vector).
Real-time polymerase chain reaction (PCR) analysis

Total RNA was extracted from lung cancer cell lines using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). It was processed directly to cDNA using a Reverse Transcription Kit (Takara, Dalian, China), followed by real-time PCR. The primer pair used for amplification of the human LncBRM gene was as follows: Forward primer, 5’-GGTCAAGAGGCCAGGAAGAG-3’ and reverse primer, 5’-TTCTCACTTCAGCCCAATGCT-3’. The human actin gene was amplified as internal control using the following primers: Forward primer, 5-GTGGACATCCGCAAAGAC-3 and reverse primer, 5-AAAGGGTGTAACGCAACTA-3. Real-time PCR included the following: 10 μl 2 × SYBR premix ex taq, 0.8 μl forward and reverse primers (2.5 μM), 5 μl cDNA, and 4.2 μl ddH₂O. The real-time PCR procedure was performed as follows: Denaturation at 95°C for 1 minute, denaturation at 95°C for 5 seconds, and annealing extension at 60°C for 20 seconds (a total of 40 cycles). All reactions were performed using the BioRad Connect Real-Time PCR platform. Absorbance values were read at the extension stage and the 2⁻ΔΔCt method was adopted for statistical analysis.

Western blotting analysis

Western blotting analysis was also performed, as previously described [15]. Briefly, the cells were lysed for total protein extraction in 2X SDS Sample Buffer (100 mM Tris-HCl [pH 6.8], 10 mM EDTA, 4% SDS, 10% Glycine). Protein extractions (20 μg) were separated using 10% SDS-PAGE and subsequently transferred to 0.22-mm nitrocellulose membranes (Sigma-Aldrich, USA). The membranes were blocked with 5% defatted milk and incubated with specific antibodies overnight. Primary antibodies against Sox4 (Ab80261) were purchased from Abcam. Primary antibodies against GAPDH (#5174) were purchased from Cell Signaling Technology. Secondary antibodies, including anti-rabbit HRP and anti-mouse HRP, were obtained from Santa Cruz Biotechnology.

CCK8 viability assay

The growth of the cells was assessed through CCK8 assays (Sigma). Equal numbers of cells were seeded in 96-well plates and cultured, for various time periods, after siRNA transfection. At indicated time points (24, 48, 72, and 96 hours), CCK8 reagent was added to the medium. The cells were incubated for another 1 hour at 37°C. Subsequently, absorbance values at 450 nm were quantitatively measured using a SUNRISE Microplate Reader (Switzerland).

Flow cytometric apoptosis analysis

A549 cells (250,000 cells/dish) were seeded in 10-cm dishes after siRNA transfection. The cells were cultured for 48 hours later (at a cell confluence of 80%), washed in PBS, and stained with Annexin V and 7-AAD (BD Biosciences, Franklin Lakes, NJ, USA) for 30 minutes. The samples were subsequently analyzed using a flow cytometer (BD LSR II).

Colonies formation assay

Equal numbers of A549 cells, treated with siControl and siLncBRM (500 cells/well), were inoculated in 6-well plates and cultured for 8 and 12 days, respectively, after siRNA infection. Subsequently, the colonies were stained using crystal violet and counted.

5-ethyl-2-deoxyuridine (EdU) incorporation assay

EdU incorporation assays were used to evaluate DNA synthesis in cells. After transfection with indicated siRNAs, the cells were incubated with 10 μM EdU for 2 hours. Incorporated EdU was detected via the fluorescent azide coupling reaction (Invitrogen). Images were captured using a fluorescence microscope (Nikon, Tokyo, Japan) and analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Mouse xenograft model

A mouse xenograft model was generated, as previously described [16]. Briefly, BALB/c athymic nude mice (6-8 weeks old) were randomly divided into two groups. They were subcutaneously injected in the flank regions with 1.0 × 10⁶ cells contained in 0.1 mL of PBS. Tumor sizes were measured every 4 days using calipers. Tumor volumes were calculated using the following formula: (length × width²)/2. Forty days after implantation, the mice were euthanized through asphyxiation in a CO₂ chamber and the tumors were immediately excised for
Moreover, Kaplan-Meier survival analysis and log-rank tests were conducted, further assessing the clinical significance of IncBRM in the prognosis of lung cancer patients. The samples were separated into high (above the median, n=35) and low (below the median, n=35) IncBRM expression groups, according to the mean level of IncBRM. Results demonstrated that increased levels of IncBRM were associated with poor OS (Figure 1B). Results also showed that IncBRM levels were correlated with histological grade (P=0.004) and TNM stage (P=0.001), but no relationship was found between IncBRM expression and other factors, including age (≤60 years or >60 years), sex (male/female), and tumor size (≤3 cm or >3 cm) (Table 1). Furthermore, univariate and multivariate analyses were conducted to confirm the prognostic role of IncBRM in patients with lung cancer. Univariate analysis identified three prognostic factors, including histological grade (low, middle, or high), TNM stage (I/II, III/IV), and expression of IncBRM (Table 2). However, other clinicopathological characteristics, such as age (≤60 years or >60 years), sex (male/female), tumor size (≤3 cm or >3 cm), and history of smoking (yes or no), were not statistically significant prognostic factors. Moreover, multivariate analysis revealed that expression of IncBRM (P=0.015), histological classification (P=0.004), and TNM stage (P=0.001) were significant independent predictors of poor survival in patients with lung cancer (Table 1). Therefore, results suggest that increased expression of IncBRM might play an important role in the development of lung cancer.

The current study determined expression levels of IncBRM in healthy lung epithelial cell line MRC-5 and lung cancer cell lines NCL-H1650, SPC-A1, HCC827, NCI-H1299, MSTO-211H, and A549. It was observed that IncBRM was highly expressed in SPC-A1, HCC827, NCI-H1299, MSTO-211H, and A549 cells. However, it was moderately expressed in NCL-H1650 cells, compared to healthy MRC-5 cells (Figure 1C). Collectively, present data suggest that IncBRM may participate in the pathogenesis of lung cancer.

**IncbRM regulates the proliferation of lung cancer cells by affecting cell cycle**

A549 cells express high levels of IncBRM. Thus, these cells were selected to perform the follow-
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In vitro loss-of-function experiments using siRNA were performed investigating the roles of lncBRM in lung carcinogenesis. Expression levels of lncBRM in A549 cells transfected with lncBRM siRNAs were significantly lower than those observed in A549 cells transfected with the scrambled sequence (Figure 2A), indicating a high knockdown efficiency. First, this study estimated the effects of lncBRM on proliferation of lung cancer cells in vitro using CCK8 assays. Intriguingly, it was found that knockdown of lncBRM significantly decreased the proliferation of A549 cells in all examined time points, indicating that lncBRM

Figure 1. IncBRM is upregulated in human lung cancer tissues and high IncBRM predicts poor prognosis. A. Relative expression levels of IncBRM in lung cancer tissues (n=70) compared with corresponding adjacent healthy tissues (n=70) using quantitative real-time PCR (qRT-PCR) assay. IncBRM expression was normalized to β-actin expression; B. Patients with high expression levels of IncBRM showed reduced overall survival times, compared with patients with low expression levels of IncBRM; C. Relative expression of IncBRM in the healthy lung epithelial cell line and various lung cancer cell lines using qRT-PCR assays. IncBRM expression was normalized to β-actin expression. *P<0.05, **P<0.01, ***P<0.001.

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<td>Sex (male, female)</td>
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<tr>
<td>History of smoking (ever, never)</td>
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<td>0.674</td>
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<tr>
<td>Tumor size (≤3 cm, &gt;3 cm)</td>
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<td>Histological grade (low, middle or high)</td>
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</tr>
<tr>
<td>IncBRM expression</td>
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<td>0.001</td>
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Abbreviation: HR, hazard ratio.
Figure 2. LncBRM regulates the proliferation of lung cancer cells by affecting the cell cycle. A. Relative expression levels of LncBRM in lung cancer cell line A549 transfected with siControl or silncBRM using quantitative real-time PCR (qRT-PCR) assay. LncBRM expression was normalized to β-actin expression; B. CCK8 assays were performed to determine the proliferation of A549 cells after transfection of siRNA against LncBRM; C. Representative results of colony formation of A549 cells after transfection of siRNA against LncBRM; D. Cell cycle was analyzed by flow cytometry after 48 hours of transfection. The bar chart represents the percentage of cells in the G1, S, or G2 phase, as indicated; E. The cells were incubated with EdU for 2 hours, then EdU positive cells were detected and counted. *P<0.05, **P<0.01, ***P<0.001.
plays a role in promoting the proliferation of these cells (Figure 2B). Similarly, results of colony formation assays showed that clonogenic survival was significantly diminished following inhibition of lncBRM in A549 cells (Figure 2C). Present results suggest that lncBRM is required for proliferation of lung cancer cells.

Subsequently, the current study investigated the roles of lncBRM in promoting the proliferation of A549 cells by affecting the cell cycle in vitro. Cell cycle analysis revealed that transfection with lncBRM siRNA resulted in a significant decrease in the number of A549 cells in the S phase and an increase in the number of cells in the G1 phase, compared with transfection with the scrambled siRNA (Figure 2D). Furthermore, EdU incorporation experimentation showed that the EdU incorporation was significantly reduced after lncBRM silencing, confirming the reduction of cells in the S phase (Figure 2E). Collectively, results suggest the increase in expression of lncBRM contributed to the proliferation of lung cancer cells by supporting cell cycle progression.

**Knockdown of lncBRM promotes apoptosis of lung cancer cells**

The current study assessed the effects of lncBRM on apoptosis using flow cytometry. As expected, the number of late apoptotic cells (Annexin V+/7-AAD+) significantly increased after knockdown of lncBRM in A549 cells (Figure 3A, 3B), indicating that lncBRM exerts anti-apoptotic effects. Moreover, the apoptosis marker cleaved caspase-3 was increased after knockdown of lncBRM in A549 cells (Figure 3C, 3D). Collectively, data suggests that lncBRM supports the growth of lung cancer by exerting anti-apoptotic effects and affecting cell cycle progression in vitro.

**Impact of lncBRM on tumorigenesis in vivo**

The above results were confirmed in vivo using a xenograft tumor model. A549 cells treated...
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with siControl and siLncBRM were subcutaneously injected into two groups of nude mice. As expected, tumor volumes in the group treated with siLncBRM were lower than those observed in the group treated with siControl (Figure 4A). In addition, the tumor growth curve showed that tumors derived from the siLncBRM group expanded more slowly than those obtained from the siControl group, at all examined time points (Figure 4B). Moreover, the current study extracted the total mRNA of tumors at the end point (day 16) to assess the efficiency of knockdown in the xenograft model. Although the efficiency was decreased, probably due to de novo mRNA production, qRT-PCR data demonstrated that levels of lncBRM remained below those reported in the control group (Figure 4C). Furthermore, immunohistochemical analysis was performed to determine expression levels of proliferation marker Ki-67 in tumor tissues. Results showed that tumors formed in the siControl group exhibited a higher Ki-67-positive signal than those formed in the siLncBRM group (Figure 4D). Results suggest that lncBRM is required for proliferation of lung cancer in vivo.

**IncBRM increases expression of Sox4**

The previously identified IncBRM target gene Sox4 [17] is closely involved in the pathogenesis of lung cancer [18, 19]. Therefore, this study examined expression levels of Sox4, aiming to investigate the mechanisms of IncBRM involved in the growth of lung cancer. Analysis showed that mRNA levels of Sox4 were reduced after silencing of IncBRM (Figure 5A). Accordingly, protein levels of Sox4 were also decreased after inhibition of IncBRM (Figure 5B). Most importantly, it was found that overexpression of Sox4 rescued inhibited cell growth caused by the silencing of IncBRM (Figure 5C, 5D). Therefore, results suggest that IncBRM may regulate the growth of lung cancer cells by affecting expression of Sox4.
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LncBRM competitively interacts with miR-211-5p and regulates Sox4 expression

It has been demonstrated that lncRNAs usually function as miRNA sponges, regulating the binding of endogenous miRNAs to their target mRNAs [20]. Thus, this study predicted 43 miRNAs with potential to bind lncBRM, using bioinformatic tools. Of these miRNA candidates, two important miRNAs, miR-204-5p and miR-211-5p, were noticed. They were predicted to directly target Sox4, according to Targetscan software (Figure 6A). Interestingly, the association between miR-204-5p and Sox4 has been previously investigated in ovarian cancer [17]. Therefore, the current study focused on miR-211-5p. First, the cells were separated into nuclear and cytoplasmic fractions, identifying the cellular localization of lncBRM. U6 and GAPDH were used as controls. Data showed that lncBRM was detected 75.1% in the cytoplasm fraction in A549 cells (Figure 6B), suggesting that lncBRM may function at the post-transcriptional level. Next, this study cloned wild-type (WT) lncBRM luciferase plasmids containing potential miR-211-5p binding sites or mutants (MUT) for each site. Luciferase assays were then performed to confirm the interaction between miR-211-5p and lncBRM after co-transfecting these plasmids with miR-211-5p mimics into 293T cells. As shown in Figure 6C, miR-211-5p mimics substantially inhibited the luciferase activity of WT lncBRM. However, they failed to affect the luciferase activity of lncBRM mutants, suggesting that miR-211-5p directly binds lncBRM. Moreover, it was found that lncBRM knockdown significantly increased miR-211-5p expression, as expected (Figure 6D).
Taken together, present results clarify the regulatory relationship between lncBRM and miR-211-5p and support miR-211-5p as an inhibitory target of lncBRM in lung cancer.

Next, the current study examined whether lncBRM regulated Sox4 expression via interacting with miR-211-5p. To test this hypothesis, this study overexpressed WT lncBRM and MUT lncBRM into A549 cells transiently. Ectopic expression of WT lncBRM, but not MUT lncBRM, significantly upregulated Sox4 expression, which was dramatically impaired after transfected with miR-211-5p mimics (Figure 6E). Furthermore, this study examined whether IncBRM regulates Sox4 expression via targeting Sox4 3'UTR. The luciferase reporter vector containing Sox4 3'UTR was co-transfected with WT or MUT IncBRM into 293T cells with or without miR-211-5p. Likewise, overexpression of WT IncBRM, but not MUT IncBRM, improved the luciferase activity of Sox4 3'UTR, while miR-211-5p totally abolished these effects (Figure 6F). In summary, results suggest that IncBRM regulates Sox4 expression in lung cancer by competitively binding miR-211-5p.
Discussion

Mammalian genomes encode large amounts of lncRNAs in addition to protein coding RNAs. Most of these lncRNAs execute important functions [4]. Currently, newly identified lncRNAs have emerged as critical factors in cellular development and human diseases. Moreover, lncBRM, first identified in liver cancer stem cells, has been shown to play a key role in the development of liver cancer [14]. However, the roles of lncBRM in lung cancer have not been examined thoroughly. The present study found that average levels of lncBRM in lung tissues were significantly higher than those observed in corresponding non-tumor tissues. In addition, the current study investigated the correlation between levels of lncBRM and clinicopathological characteristics, as well as prognosis, in patients with lung cancer. It was found that high expression of lncBRM in lung cancer tissues was associated with poor prognosis, suggesting that it may be an independent prognostic indicator. These findings suggest that lncBRM may play a critical role in the development and progression of lung cancer. The RNA interference approach was used to further analyze the roles of lncBRM in the development of lung cancer. Inhibition of lncBRM in A549 lung cancer cells suppressed the growth of lung cancer cells in vitro. These effects were confirmed using an in vivo xenograft tumor model. Moreover, this study revealed that lncBRM participated, at least partially, in these processes by regulating expression levels of the gene Sox4. Collectively, present data demonstrate the key roles of lncBRM in lung cancer.

Cancer cells possess the ability to uncontrollably proliferate and resist cell death [21]. An important finding of this study was the identification of lncBRM as a key participator in lung cancer by altering the proliferation and apoptosis of cancer cells. It was found that silencing of lncBRM induced evident arrest at G0/G1 and a decrease in the number of cells at the S phase. Results suggest impaired cell cycle progression. Using flow cytometry, knockdown of lncBRM induced more apoptosis in lung cancer cells. This may contribute, in part, to the reduced cell growth. Present results are consistent with those reported in a previous study investigating liver cancer, suggesting that the roles of lncBRM are relatively conserved in different types of cancer. However, the exact mechanisms regulating these processes are unclear. Moreover, the effects of lncBRM silencing on expression of apoptosis-related proteins (Bcl2, Bax) have yet to be determined.

Sox4, a member of the Sry-related high mobility group box (Sox) family of transcription factors, has been considered a master regulator in tumorigenesis and cancer stemness [22, 23]. Numerous studies have demonstrated that expression of Sox4 is increased in various cancers (lung, colorectal, prostate, and esophageal) [19, 24-26]. The present study demonstrated that knockdown of lncBRM reduced levels of Sox4 mRNA and protein, suggesting that Sox4 may be a downstream target gene regulated by lncBRM. More importantly, overexpression of Sox4 rescued, at least in part, impaired cell growth caused by lncBRM silencing. These findings indicate that Sox4 participates in the process of cell growth affected by lncBRM in lung cancer. Present data are consistent with that of a previous study investigating ovarian cancer [17]. The molecular mechanisms of lncRNAs mainly function as: (1) Decoys to locate transcription factors; (2) Regulatory signals for transcription; (3) Scaffolds to aggregate different proteins; (4) A ‘sponge’ to interact with microRNAs; and (5) Guides to binding to specific proteins to target genes [27]. The current study found that lncBRM may function as a ‘sponge’ to interact with miR-212, leading to the reduction of miR-212 levels in lung cancer cells. Decreased miR-212 then abolishes the inhibitory effects to Sox4, eventually causing lung cancer development.

In summary, the present study demonstrated that high expression of lncBRM confers malignant growth in lung cancer cells. This occurs mainly by affecting proliferation and inhibiting apoptosis in vitro and in vivo. Current findings suggest that lncBRM might be a biomarker for progression of lung cancer. Moreover, present results suggest that lncBRM might be a tumor-promoter gene in certain types of cancer, paving the way for the development of new therapeutic modalities. Lastly, lncBRM depletion may be a promising strategy for treatment of lung cancer.

Disclosure of conflict of interest

None.
References


[15] Li BH, He FP, Yang X, Chen YW and Fan JG. Steatosis induced CCL5 contributes to early-stage liver fibrosis in nonalcoholic fatty liver disease progress. Transl Res 2017; 180: 103-117, e104.


