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Research article

# Down regulation of RBM10 promotes proliferation and metastasis via miR-224-5p/RBM10/p53 feedback loop in lung adenocarcinoma

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ABSTRACT

RNA-binding motif protein 10 (RBM10) has a tumor suppressor role in multiple cancers. Combining Oncomine database results with tissue samples, Western blot analysis showed that RBM10 was significantly lower in lung adenocarcinoma (LUAD) than in adjacent normal tissues. Moreover, KM analysis revealed that the group with higher RBM10 expression in LUAD correlated with better overall survival (OS). Luciferase reporter assay revealed that an important tumor-promotive miRNA, miR-224-5p, was directly bound to the 3′UTR of RBM10, resulting in inhibition of RBM10 expression, and promoted LUAD progression both in vitro and in vivo. Mechanistically, we found that miR-224-5p directly targeted RBM10 to inhibit p53 expression during LUAD progression. Meanwhile, p53 affected RBM10 expression through p53/miR-224-5p axis. Our study identified RBM10 as a key tumor suppressor in the proliferation and metastasis of LUAD. The findings provide a novel mechanism involving a feedback loop of miR-224-5p/RBM10/p53 regulated tumor progression in LUAD, which may help with the design of more effective LUAD treatments.

## 1. Introduction

LUAD is often diagnosed at an advanced stage, and its prognosis remains poor [1]. Therefore, a better understanding of the mechanisms underlying the carcinogenesis and progression of LUAD is required. RBM10 is one of the most well-known cancer-related RNA binding protein (RBP) [2,3]. Several initial functional studies associated RBM10 expression with the regulation of mRNA stabilization, increased apoptosis, decreased cell proliferation, alternative splicing, nuclear output, decreased colony formation, cell cycle arrest, translation, and decreased xenograft tumor growth [4]. In our previous study, we found that RBM10 inhibited epithelial mesenchymal transition at least partly by negatively regulating the Wnt/β-catenin pathway [5]. However, most recent studies have suggested a tumor-promoting role for RBM10. This functional dichotomy highlights the importance of obtaining a deeper insight into the downstream effects of RBM10 expression, and the mechanisms regulating RBM10 itself.

MicroRNAs (miRNAs) are a class of small, non-coding RNAs that negatively regulate the expression of a wide variety of genes by binding to complementary sequences in the 3′-untranslated regions (UTRs) of target mRNAs [6]. A lot of research confirmed that miRNA alteration or dysfunction result in cancer development and progression by regulating cancer angiogenesis, apoptosis, differentiation, metastasis, metabolism, and cell proliferation [7–11]. The aberrant expression of miRNAs were involved in lung

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carcinogenesis and progression [12–14]. The miR-224-5p plays important tumor-promoting roles. In thyroid carcinoma miR-224 promoted the development of papillary cancer by targeting early growth response protein 2 (EGR2) [15]. In hepatocellular carcinoma, miR-224 suppressed homeobox D 10 (HOXD10) expression result in cell migration and invasion [16]. MiR-224 played a vital role in lung cancer, which was significantly upregulated in lung cancer tissues compared with normal adjacent tissues, resulting in migration, invasion, and proliferation of lung cancer cells [17,18]. MiR-224-enriched exosomes promoted tumorigenesis by directly targeting androgen receptor in non-small cell lung cancer (NSCLC) [19]. MiR-224 suppressed angiopoietin-like protein (ANGPTL1) expression and promoted lymphatic metastasis in NSCLC [20]. These results suggest that lung cancer progression was profoundly affected by miR-224 expression. However, the roles of miR-224 in lung cancer are not fully understood.

Combined with bioinformatics analysis, we found that RBM10 was downregulated in LUAD and associated with OS. Subsequent studies showed that RBM10 inhibited LUAD proliferation and migration and was a target gene for miR-224-5p. Further studies showed that miR-224-5p promoted LUAD proliferation and migration in vitro and accelerated xenograft tumor growth in vivo by targeting RBM10. RBM10 was a regulator of p53. Further, the transcription factor p53 promoted RBM10 expression by inhibiting miR-224-5p. Therefore, our study revealed the presence of a miR-224-5p/RBM10/p53 feedback loop in the progression of LUAD.

## 2. Materials and methods

### 2.1. Online database analysis

The Oncomine database (http://www.oncomine.org) was utilized to analyze the degree of RBM10 expression in normal lung tissues and LUAD. We used the Kaplan-Meier plotter (http://kmplot.com) database to assess the association between RBM10 expression and patient survival. Targetscan (http://www.targetscan.org/vert_71/), miRanda (http://www.microrna.org/), YM500 (http://ngs.ym.edu.tw/ym500/) and miRWalk (http://129.206.7.150/) were used to predict potential miRNAs that target RBM10.

### 2.2. Human LUAD tissues

The clinical specimens were collected from patients diagnosed with lung adenocarcinoma (LUAD) and followed patients' informed consent. These were all approved in previous studies including adjacent normal lung tissues (N1-5) and LUAD (T1-5), from Harbin Medical University [5].

### 2.3. Cell culture and transfection

Human bronchial epithelial (HBE), and human LUAD cell lines H1299, H1993, A549 were obtained from American Type Culture Collection (ATCC) and approved in previous studies [5]. Cells were cultured in RPMI-1640 medium supplemented with 10 % FBS (Gibco). miR-224-5p mimics, inhibitors, small-interfering RNAs (siRNAs) targeting p53, and a negative siRNA control (si-NC) (Ribobio, Guangzhou, China), were transfected using Lipofectamine 2000 (Invitrogen, CA, USA). Protein overexpression was accomplished with gene-specific overexpression constructs (Genechem, shanghai, China) [16].

### 2.4. Cell proliferation assays

Briefly, cell viability was determined by using the Cell Counting Kit-8 (CCK-8) (SEVEN Biotech, Beijing, China). $5 \times 10^3$ cells/well were plated into a 96-well plate and examined on 24 h at 450 nm absorbance. In addition, the EdU Kit (RiboBio, Guangzhou, China) was conducted basing on the manufacturer's protocol to detect cell proliferation by fluorescence microscopy [5].

### 2.5. Wound healing assay

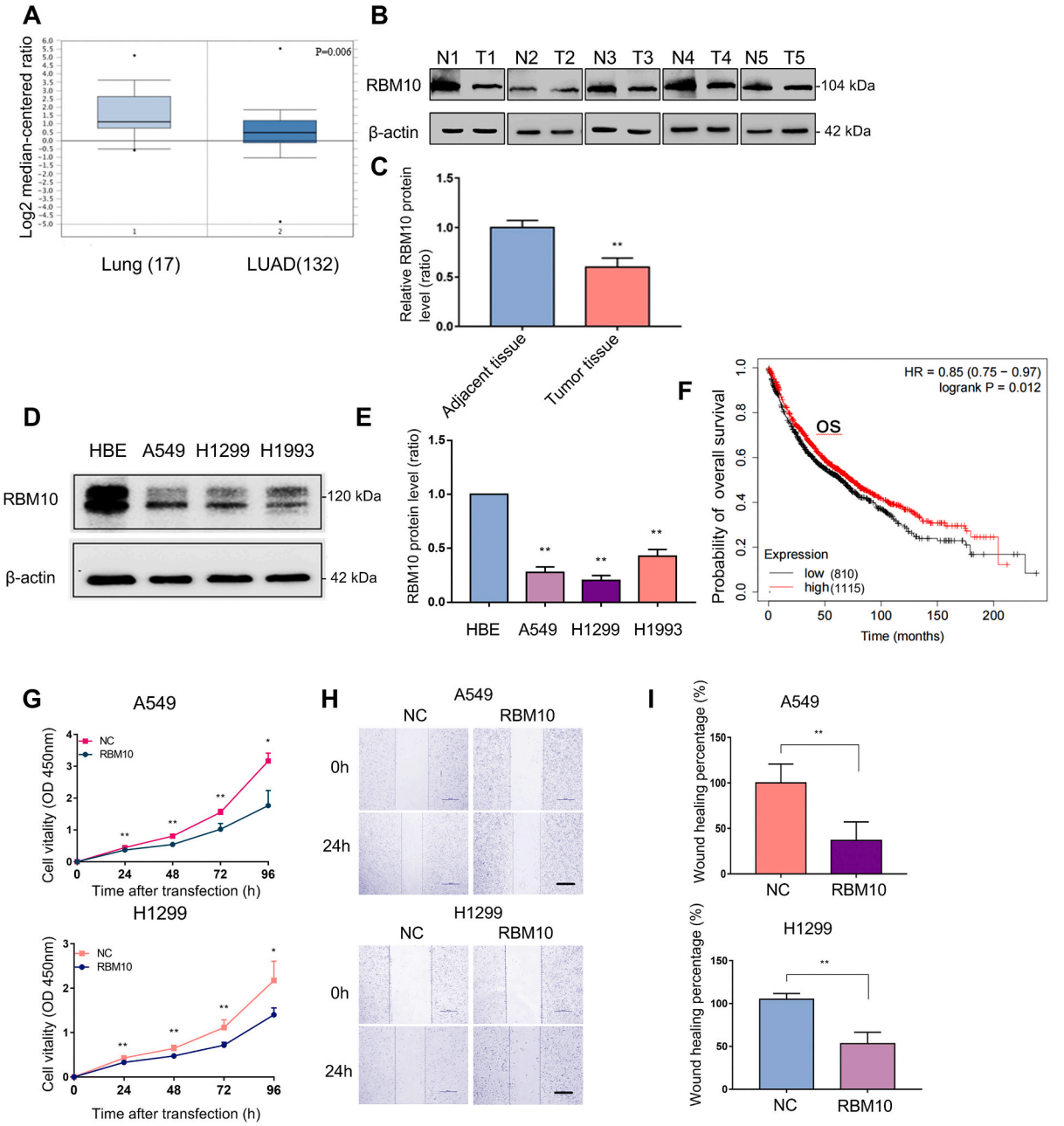
In this assay, the monolayer cells were scratched using a pipette tip to create liner wounds when the cells density reached over 80 % in 6-well plates. The speed of wound healing was observed. Microscope (Olympus, Tokyo, Japan) was used to capture images [15].

### 2.6. Transwell assay

Briefly, the Transwell chamber (BD Biosciences, New Jersey, USA) was placed on a 24-well plate. Cell suspension was added to the upper chamber coated with diluted Matrigel (BD Biosciences, New Jersey, USA), and the lower chamber was added with 700 μL medium. After 24 h or 48 h, paraformaldehyde was added to fix for 30 min and stained with crystal violet. Finally, cells were counted in 5 random fields and photographed under a microscope (Leica Microsystems Inc., USA) [20].

### 2.7. Protein isolation and western blot

Antibodies against RBM10, β-actin and p53 were purchased from CST (#30774), Origene (TA329114), Origene (TA811000S), Abcam (Ab26). Protein isolation and western blotting was performed as described elsewhere [17].




A. RBM10 levels of LUAD compared with normal lung samples based on the Oncomine database (http://www.oncomine.org). B-C. RBM10 expression in five pairs of fresh human LUAD and normal adjacent tissues. D-E. Western blot analysis of RBM10 levels in HBE and A549, H1299, and H1993. F. Kaplan-Meier plotter database (http://kmplot.com) was searched for the overall survival of LUAD patients. Patients were stratified into groups of "low" and "high" RBM10 RNA expression, based on the auto-select best cutoff. HR: hazard ratio. G-I. RBM10 was overexpressed in A549 and H1299. G. Cell proliferation was analyzed with CCK-8; H-I. A wound healing assay was used to test the migration capacity of RBM10 in LUAD cells. The cells migrating into the wounded areas were photographed at 0h and 24h. The results were expressed as mean ± SD, *P < 0.05, **P < 0.01, Scale bar: 100 μm.

**Fig. 1.** Low RBM10 expression is associated with poor prognosis in LUAD.

### 2.8. RNA isolation and quantitative reverse transcription-PCR (qRT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen, USA). A Fast Quant RT Kit (TIANGEN, Beijing China) and miRNA First-Strand cDNA Synthesis (Tailing Reaction) Kit (Sangon Biotech, Shanghai, China) were used for reverse transcription of protein-coding genes and miRNAs, respectively. qRT-PCR was performed using StepOnePlus Real-time PCR instrument with MicroRNAs qPCR Kit (SYBR Green; Sangon Biotech, Shanghai, China) and Talent qPCR Pre-Mix Kit (SYBR Green; TIANGEN, Beijing, China). The level of specific mRNA expression was quantified using the $2^{-\Delta\Delta CT}$ method. U6 snRNA and GAPDH were used as internal controls for miRNAs and protein-coding genes, respectively. The primers are listed in the Additional file 1: Table S1 [19].

### 2.9. RIP assay

The Magna RIP RNA-binding protein immunoprecipitation kit (Millipore, MA, USA) was used for the RIP experiment. The cell lysate was incubated with RIP buffer containing magnetic beads bound with normal mouse IgG (Millipore, MA, USA) or human anti-Argonaute2 (Ago2) antibody (Abcam, USA). Finally, co-immunoprecipitated RNA was amplified by qRT-PCR and analyzed [44].

### 2.10. Luciferase assay

Briefly, miRNA binding site tests were constructed using pSI-Check2 constructs containing binding sites for miR-224-5p on RBM10's 3′UTR. The miR-224-5p binding site was mutated from GUGACUU to AUUAUUC (Hanbio Biotechnology, Shanghai, China) [22].

### 2.11. Tumor xenograft model in nude mice

A xenograft model was constructed, by injecting $2.5 \times 10^7$ cells/ml A549 cells (150 μl/injection) (A549-construct, A549-miR-224, A549-RBM10, A549-miR-224-RBM10). The tumor tissues were collected, weighed, soaked in formalin, embedded in paraffin for hematoxylin and eosin (H&E) staining and immuno-histochemistry (IHC) analysis and approved in previous studies [5,29].

### 2.12. IHC and H&E staining

5-μm thick longitudinal sections were stained in hematoxylin solution for 3min, and then reacted with hydrochloric acid ethanol until the nuclei were blue. Subsequently, the sections were added with eosin. Based on the standard method, studies were performed using formalin-fixed, paraffin-embedded tissue sections. Primary antibodies used for incubation in IHC analysis, Ki67 (A2094, Abclonal, 1:100), and RBM10 (TA329114, Origene, 1:200). Eventually, the tissue sections were imaged using a microscope (Olympus, Toyo, Japan). The intensity of specific staining was measured using Image-Pro Plus 6.0 software (Media Cybernetics, Silver Spring, MD, USA). The positive staining intensities were determined using the mean integrated optical density (mean IOD) [5,27].

### 2.13. Co-immunoprecipitation (Co-IP)

To summarize, the 500 μL of A549 cells lysate were incubated with 2 μg of the antibody at 4 °C overnight. Then, protein A/G magnetic beads were added to bind antigen-antibody complex, followed by washing the beads with cold PBS via centrifugation at 3000×*g* for 2 min. Finally, the bound antigen was eluted [5].

### 2.14. Statistical analysis

GraphPad Prism 7.0 software was performed to analyze the data. The data was displayed as mean ± SD, unless stated otherwise. The difference between two group was analyzed by *t*-test, whereas ANOVA was used for more than two groups. P value $< 0.05$ was considered statistically significant.

## 3. Results

### 3.1. RBM10 was significantly downregulated and acted as an anti-oncogene in LUAD

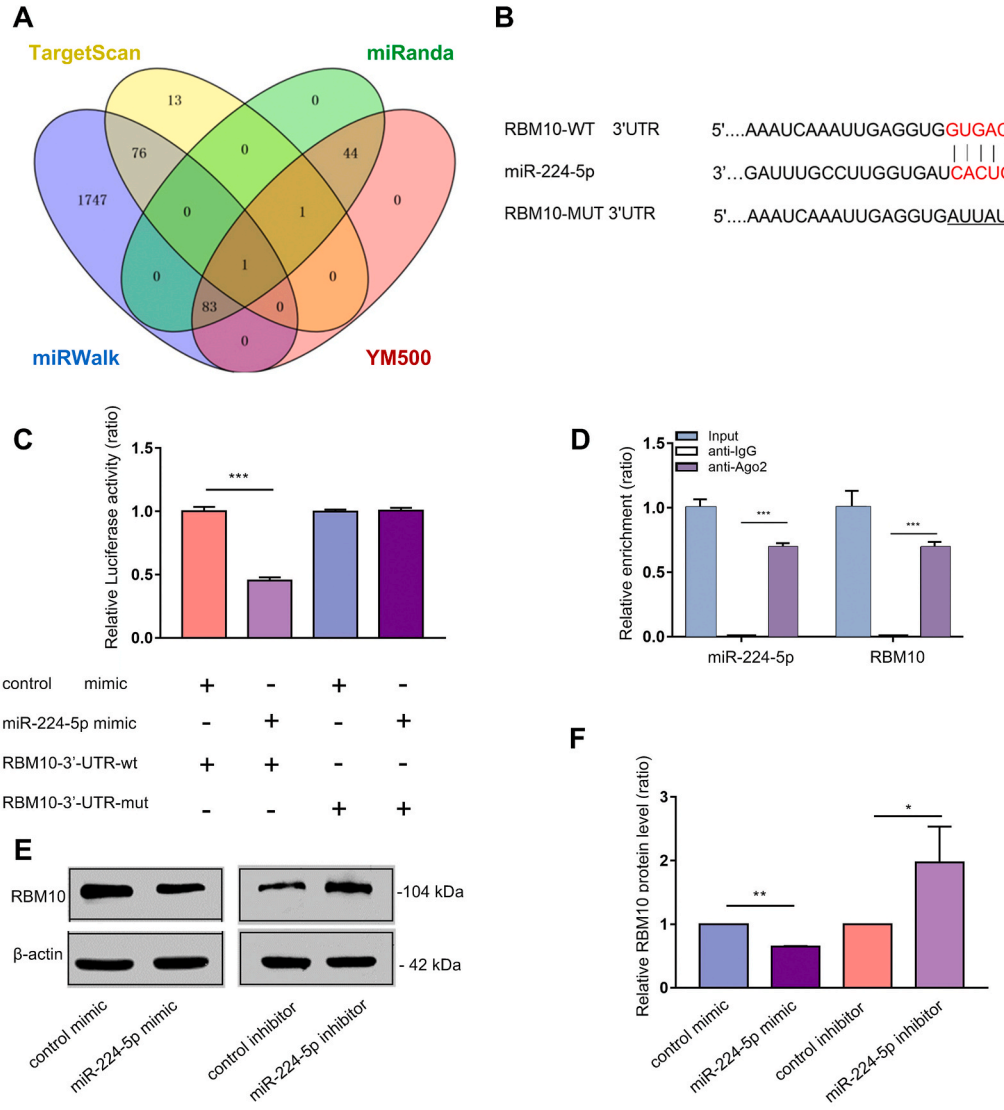
Based on the Oncomine database, we found that the expression of RBM10 in LUAD was decreased compared with that in normal lung tissues (Fig. 1A). This result was validated using 5 pairs of LUAD and adjacent normal tissues, in which RBM10 protein levels were significantly downregulated compared with levels in normal tissues (Fig. 1B and C) ($P = 0.0031$). Consistently, RBM10 was significantly downregulated in LUAD cell lines (A549, H1299 and H1993) compared with normal lung epithelial cell by Western blot (Fig. 1D and E). Additionally, KM analysis revealed that the group with higher RBM10 expression correlated with better OS (Fig. 1F). Overall, these results suggest an anti-oncogenic role of RBM10 in LUAD.

To investigate whether RBM10 was involved in LUAD proliferation and metastasis, RBM10 was overexpressed in the LUAD cell lines A549 and H1299 by transfecting RBM10 overexpressing lentivirus constructs. After the overexpression efficiencies were determined by Western blot (Fig. S1A). RBM10 overexpression significantly reduced the growth of A549 and H1299 cells by using

CCK-8 (Fig. 1G). A wound healing assay was performed to investigate the effect of RBM10 on tumor cell migration. The healing ability of the cells in the groups overexpressing RBM10 (A549 and H1299 cells) was also significantly lower than in the control group (Fig. 1H and I). Overall, our results suggested that RBM10 suppressed LUAD proliferation and metastasis in vitro.

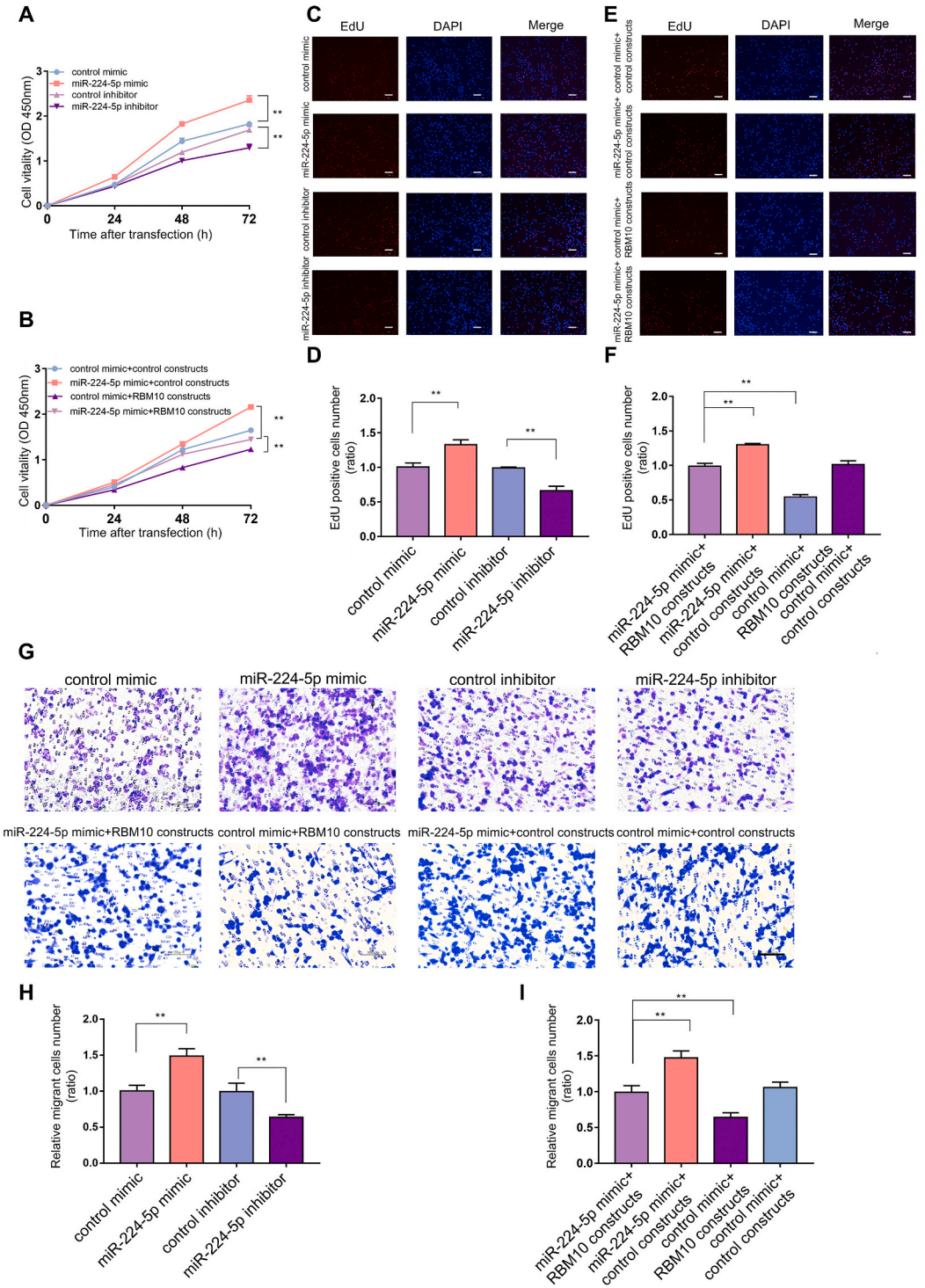
### 3.2. RBM10 was a potential target of miR-224-5p

To investigate the causes of downregulated RBM10 in LUAD. We hypothesized that some miRNAs might target RBM10 in LUAD. First, the bioinformatic software, including Targetscan, miRanda, miRwalk, and YM500 were used to explore the candidate miRNAs that might target RBM10 by examining sequence complementarity. Target predicted by at least three algorithms were selected for the following studies. By sorting the overlap results, miR-224-5p was chosen for further verification (Fig. 2A). As displayed in Fig. 2B, miR-



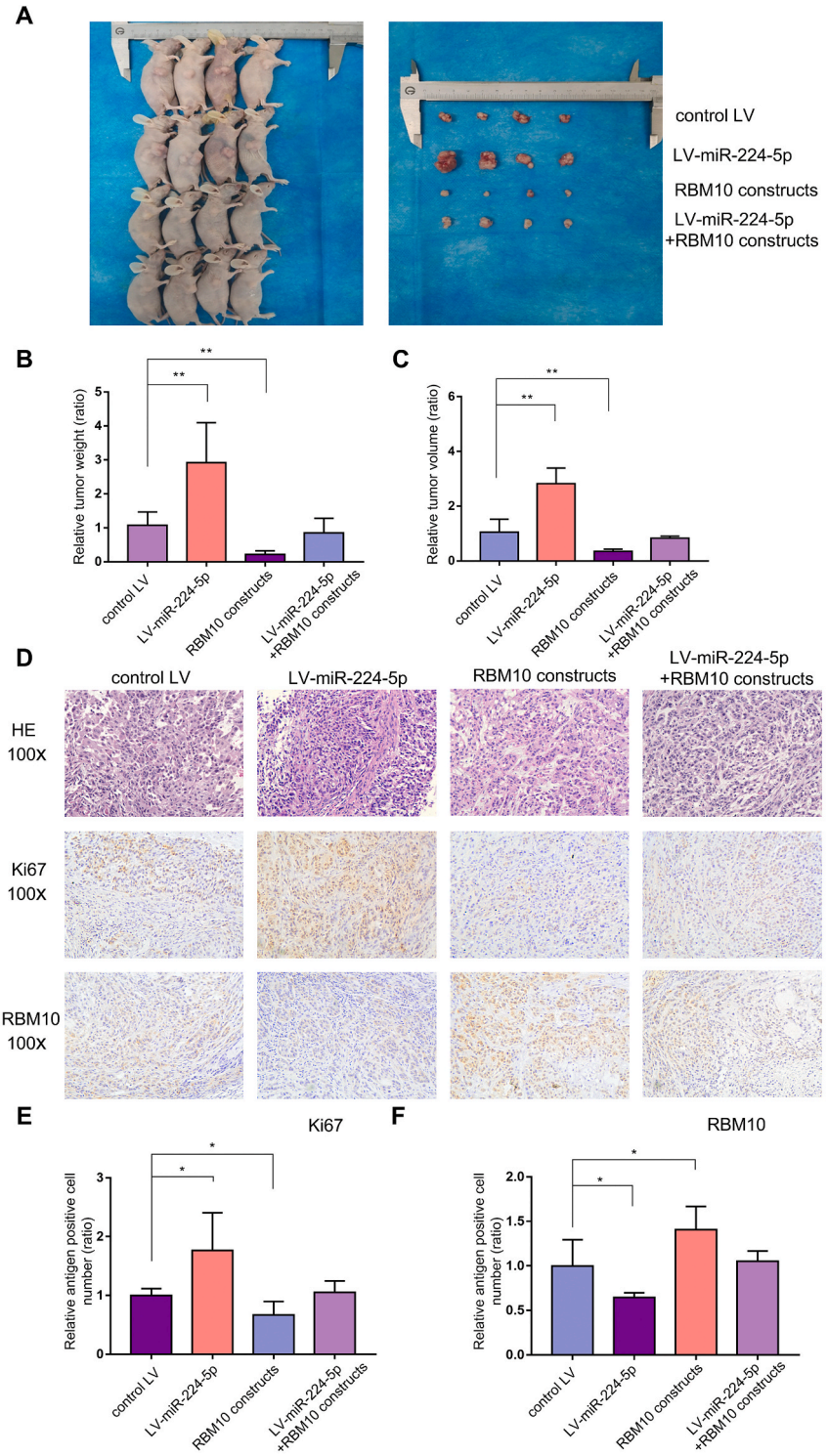

A. Venn diagRAms show the candidate genes targeting RBM10 using prediction algorithms, including TargetScan, miRanda, miRWalk and YM500. B. The putative wild-type or mutant miR-224-5p binding sites in the 3'UTR of RBM10 mRNA. C. Luciferase reporter assay showed that RBM10 is one of the target genes of miR-224-5p. D.RIP assay with Ago2 antibody was used to confirm the binding status of miR-224-5p and RBM10 mRNA. E-F. Western blot analysis of RBM10 protein expression in A549 cells after transfection with miR-224-5p mimic, miR-224-5p inhibitor, or miR-control. ***P < 0.001.

**Fig. 2.** Evidence of inverse regulation of RBM10 by miR-224-5p.




A, C-D. miR-224-5p promotes A549 proliferation. A. CCK-8 assay; C-D. EdU assay. B, E-F. Recovery experiments indicated that the promotion of A549 proliferation by miR-224-5p was mediated by inhibition of RBM10 expression. B. CCK-8 assay; E-F. EdU assay. G-H. Transwell assays revealed that miR-224-5p accelerated A549 migration. G, I. Recovery experiments indicated that the promotion of A549 migration by miR-224-5p was mediated by its inhibitory effect on RBM10.

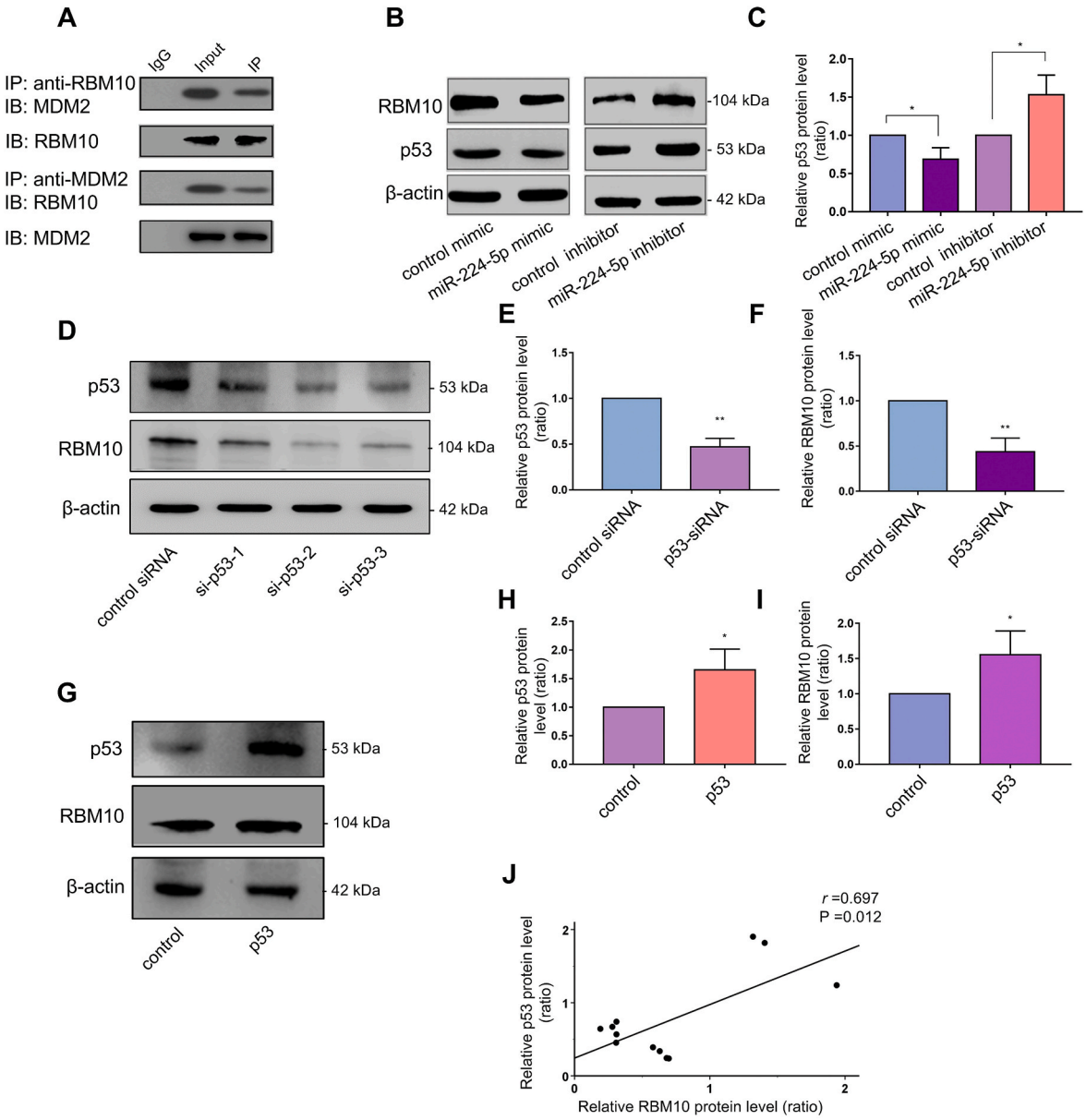
**Fig. 3.** miR-224-5p promoted A549 proliferation and migration in vitro by targeting RBM10.




A-C. miR-224-5p promoted LUAD xenograft tumor growth. A. Representative images of detached tumor sections; B. Tumor weights; C. Tumor volume. D-F. Representative images of H & E staining and IHC of RBM10 and Ki-67 expression in nude mice xenograft tumor sections (magnification, × 100). n = 6, * P < 0.05, **P < 0.01.

**Fig. 4.** miR-224-5p promotes LUAD tumor growth in vivo by targeting RBM10.

224-5p was predicted to contain the potential binding sites for 3′UTR of RBM10, which garnered our attention. Luciferase reporters containing wild-type or mutant miR-224-5p binding sites in RBM10 3′UTR were constructed and co-transfected with miR-224-5p or miR-control into 293T cells to confirm whether miR-224-5p directly targeted RBM10. The results showed that compared with the control group, co-transfection with miR-224-5p and WT 3′UTR of RBM10 significantly reduced luciferase activity ($P < 0.001$); however, transfection of miR-224-5p and MUT 3′UTR of RBM10 had no obvious effect on luciferase activity (Fig. 2C). To determine that the binding status of miR-224-5p and RBM10 mRNA, we further performed RIP assay of A549 cell extracts using specific antibody




A. co-IP-IB assays were used to validate the interaction between RBM10 and MDM2 in A549 cells. RBM10 was pulled down by anti-RBM10. Western blot Was used to detect MDM2, RBM10. MDM2 was pulled down by anti-MDM2, followed by detection of RBM10 and MDM2 by western blot. B-C. Western blot analysis of p53 levels following altered miR-224-5p expression or activity in A549 cells. D-I. Western blot analysis of RBM10 levels following altered p53 expression or activity in A549 cells. J. Pearson's correlation scatter plot of RBM10 and p53 protein levels in A549 cells.

**Fig. 5.** Feedback loop of miR-224-5p/RBM10/p53 exists in A549 cells.

against Ago2 protein, which is a key component of RNA-induced silencing complex (RISC) mediating miRNA-induced mRNA degradation or translational repression. As shown in Fig. 2D, both miR-224-5p and RBM10 mRNA were enriched in Ago2 coating beads compared to IgG control group ($P < 0.001$). The interactions among miR-224-5p, RBM10 were successfully validated. In addition, the levels of miR-224-5p in A549 cells were altered by transfecting miR-224-5p mimic or inhibitor (Additional file 1: Fig. S1B). Overexpression of miR-224-5p significantly reduced the RBM10 protein level in A549, while downregulation of miR-224-5p leads to RBM10 upregulation (Fig. 2E and F). We repeated this experiment in another LUAD cell line, H1299, and observed similar results (Fig. S1D). The alteration of RBM10 mRNA levels after transfected with miR224-5p mimic and inhibitor was shown in Fig. S1C. The above findings showed that miR-224-5p directly targeted RBM10 and inhibited RBM10 expression in A549 cells.

### 3.3. *miR-224-5p promoted A549 proliferation and migration in vitro by targeting RBM10*

To determine the regulation of A549 cell proliferation by miR-224-5p. Overexpression of miR-224-5p accelerated A549 proliferation, whereas downregulated miR-224-5p resulted in an opposite effect by CCK-8 assay (Fig. 3A).

The EdU assay showed similar results (Fig. 3C and D). Transwell assays also revealed that the overexpression of miR-224-5p promoted A549 cell migration, which was inhibited by reduced miR-224-5p expression (Fig. 3G and H).

To determine if the promotion of proliferation and migration of A549 by miR-224-5p were due to the targeting of RBM10 with miR-224-5p, we performed recovery experiments in which an RBM10 overexpression construct was used to specifically restore RBM10 expression suppressed by miR-224-5p. The restoration of RBM10 in A549 abolished the proliferation by miR-224-5p (Fig. 3B). In EdU assays, the RBM10 construct evidently reduced the A549 cell proliferation, which was enhanced by miR-224-5p (Fig. 3E and F). Significantly, restoration of RBM10 expression resulted in a lower percentage of migrant cells compared with that in the miR-224-5p mimic group in Transwell assays (Fig. 3G–I). Taken together, these results confirmed that miR-224-5p functioned as a tumor-promotive miRNA to enhance A549 proliferation and migration by targeting RBM10.

### 3.4. *miR-224-5p promoted LUAD tumor growth in vivo by targeting RBM10*

To validate the effect of miR-224-5p induced RBM10 inhibition on LUAD tumorigenesis in vivo, we injected A549 cells overexpressing miR-224-5p and/or RBM10 into the armpits of nude mice to construct a xenograft model of LUAD. The growth curve of xenografted tumors showed that overexpression of RBM10 delayed tumor growth, whereas miR-224-5p markedly promoted it. Restoration of RBM10 reversed the promotion of tumor growth by miR-224-5p (Fig. S1E). The mice were sacrificed, and the tumors were removed and weighed. As shown in Fig. 4A, B, and C, miR-224-5p overexpression promoted xenograft tumor growth ($P < 0.01$), whereas RBM10 significantly attenuated this process ($P < 0.01$). Restoration of RBM10 diminished the tumor-promotion by miR-224-5p. H&E staining and IHC analysis of xenografts to determine RBM10 and Ki-67 expression revealed that RBM10 levels were diminished and higher percentage of proliferative cells were found in LV-miR-224-5p infected tumors ($P < 0.05$). In contrast, tumors overexpressing RBM10 showed fewer proliferative cells than the control group ($P < 0.05$). RBM10 restoration decreased the proliferation rate enhanced by LV-miR-224-5p (Fig. 4D, E, and F). These results revealed the tumor-promotive role of miR-224-5p in vivo mediated by targeting RBM10.

### 3.5. *The feedback loop of miR-224-5p/RBM10/p53 regulated LUAD progression*

RBM10 could competitively binds with MDM2 and inhibits p53 degradation by disrupting MDM2-p53 interaction [3,48]. We tested if RBM10 might interacted with MDM2 in A549 cells, by conducting a set of reciprocal co-IP-IB assays. As shown in Fig. 5A,

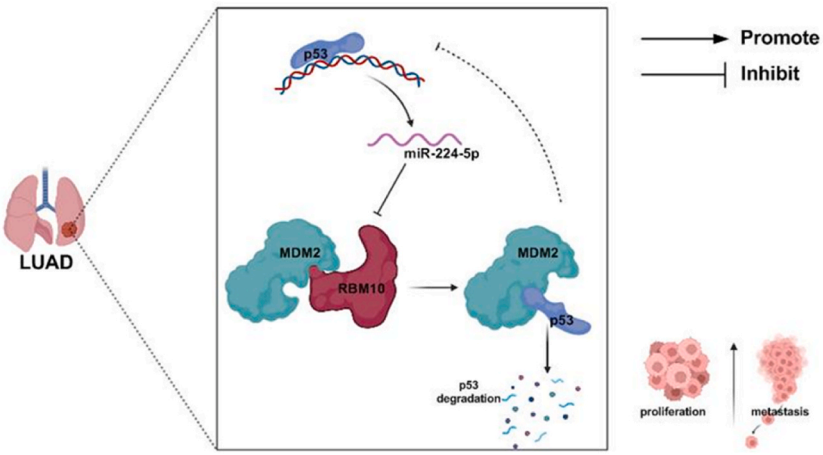



**Fig. 6.** The schematic diagram to visualize this feedback loop.

endogenous RBM10 bound to endogenous MDM2, and vice versa. Consistent with this result, ectopic miR-224-5p also reduced the protein levels of p53, whereas knockdown of miR-224-5p increased the protein levels of p53 (Fig. 5B and C). And p53 mRNA levels were not significantly altered after transfected with miR224-5p mimic and inhibitor (Additional file: Fig. S1F). Taken together, these results demonstrated that miR-224-5p suppressed p53 by inhibiting RBM10-MDM2. Studies have shown that p53 could inhibits miR-224-5p transcription, thereby reducing the expression of miR-224-5p [21–23]. We tested pri-miR-224-5p, miR-224-5p levels by a qRT-PCR assay when p53 overexpressed and silenced, the results were shown in Figs. S1G–H. Next, we investigated whether p53 indirectly affected RBM10 expression in A549 cells. As shown in Fig. 5D, E, and F, inhibition of p53 expression decreased RBM10 expression while overexpression of p53 increased RBM10 level (Fig. 5G, H, and I). Pearson correlation analysis revealed a significant positive correlation between the expression of p53 and RBM10 (Fig. 5J). These results indicated that the alteration of RBM10 levels by p53 may occur partially through the p53-miR-224-5p axis.

Prior studies had reported that RBM10 was a regulator of p53 [3]. RBM10 overexpression prolonged the half-life of p53 by disrupting MDM2-p53 interaction and subsequently repressing p53 degradation [3,24–26]. These findings, combined with our results, support the direct binding of miR-224-5p to RBM10 3′UTR, and downregulation of RBM10 expression. Further, decreased expression of RBM10 promoted MDM2-mediated ubiquitination and degradation of p53 via binding with MDM2 directly. Finally, p53 degradation, which in return upregulates miR-224-5p expression and downregulates RBM10 expression (Fig. 6). Moreover, RBM10 silencing promoted proliferation and metastasis in LUAD. In summary, these results indicated the existence of a feedback loop involving miR-224-5p/RBM10/p53 in LUAD.

## 4. Discussion

RBM10 is a representative RNA binding protein that plays a vital role in multiple cancers [4,27,28]. Recent discoveries revealed that RBM10 expression is downregulated in a substantial proportion of cancers [29–31]. However, the specific molecular mechanism underlying the regulation of RBM10 in cancer proliferation and metastasis remained largely unknown. In this study, we presented evidence for the first time demonstrating that the molecular mechanisms of downregulated RBM10 in LUAD correlated positively with cancer proliferation and metastatic features. Further, we found that forced RBM10 expression in LUAD cells reduced proliferation and metastasis in vitro, resulting in significant inhibition of tumor growth in vivo.

The present study highlighted the mechanism of RBM10 downregulation in LUAD. Our findings suggest that the downregulation of RBM10 in tumor cells is a major cause of increased lung cancer proliferation and metastasis. The expression of RBM10 in LUAD and adjacent normal lung tissues were analyzed using bioinformatics databases. As well, the impact of RBM10 on the prognosis of patients with LUAD was determined. We found that RBM10 protein expression was relatively low in LUAD compared with adjacent normal tissues. Survival analysis showed that the group with higher RBM10 expression correlated with better OS. Our findings are consistent with most previous studies involving RBM10 [29,32,33]. In this study, RBM10 suppressed LUAD cell proliferation and migration in vitro and reduced LUAD tumor growth in vivo. MiR-224-5p was found to be an upstream repressor of RBM10 mediated via direct binding to its 3′UTR.

The miRNAs alter the cell expression in multiple phases and events of tumorigenesis [34–36]. Among the tumor-related miRNAs, miR-224-5p affects various tumor phenotypes, including proliferation, migration, invasion, immune escape, apoptosis and angiogenesis [16,20,37,38]. Here, miR-224-5p acted as a tumor-promoting miRNA in LUAD by accelerating the proliferation, migration and tumor growth by targeting RBM10. Studies have shown that miR-335 also interacted with RBM10 in endometrial cancer to modulate alternative splicing of Numb [39]. Our results highlighted the interaction between miR-224-5p and RBM10 in LUAD, and suggested a new treatment strategy by targeting miR-224-5p or RBM10 in clinical environments [40–43].

Studies investigating RBM10 mainly focused on alternative splicing and RBM10 regulatory pathways, such as RBM10 regulated human TERT gene splicing and inhibition of pancreatic cancer progression [44]; RBM10 regulated alternative splicing of VEGFA in endometrial cancer [31]; RBM10 inhibited cell proliferation of LUAD via the RAP1/AKT/CREB signaling pathway [30]; and RBM10-mediated repression of tumor progression via Wnt/β-catenin pathway in LUAD [5]. Despite these studies, which were significant, the cause of RBM10 downregulation in LUAD was unknown. As regulators of gene expression, transcription factors (TFs) and miRNAs undergo complex regulation in cancers. The role of TF/miRNA/gene regulatory axis in cancer progression has been discovered, such as regulation of tumor immune evasion via p53/miR-34/PDL1 axis and C-myc/miR-150/EPG5 axis mediated dysfunction of autophagy, which promoted the development of NSCLC [45,46]. Various upstream regulatory mechanisms of genes have been elucidated. In this study, we demonstrated the regulation of LUAD via the miR-224-5p/RBM10/p53 pathway. Preliminary studies demonstrated the tumor suppressor role of RBM10, however, conflicting results suggested a tumor promoter role as well [4,47]. In this study, we demonstrated that RBM10 up-regulated the expression of p53 in LUAD, which was consistent with the findings of Jung et al. [48]. P53 is a well-known tumor suppressor, thus, our study validated the tumor suppressive effect of RBM10 in LUAD simultaneously. Prior studies showed that p53 directly inhibited the transcription of miR-224-5p and its host gene [21–23]. This finding, combined with our results, revealed that p53 indirectly increased the levels of RBM10 expression by inhibiting miR-224-5p.

Overall, this study identified RBM10 as a key tumor suppressor in the proliferation and metastasis of LUAD. The findings may provide a novel mechanism involving a feedback loop of miR-224-5p/RBM10/p53 regulated tumor progression in LUAD. Our study offers an expanded therapeutic window compared with direct miR-224-5p inhibition, since they are required for normal cellular homeostasis. And there are still some potential limitations to the above results, such as add multi-omics analysis. In addition, many aspects need to be studied further, for instance exploring additional regulatory pathways involving RBM10 or investigating the efficacy of targeting miR-224-5p/RBM10/p53 in clinical trials. In all, these findings provide new insight into the potential anti-oncogenic role of RBM10 in LUAD progression, rendering it as a potential biomarker or even therapeutic target to prolongate survival time.

## Funding

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## Ethics statement

All procedures were approved by the Harbin Medical University Cancer Hospital (KY2019-19). The animal experiments were approved by the Ethics Committee of Harbin Medical University Cancer Hospital (NO.:KY2017-12). These were all approved in previous studies [5].

## Data availability statement

Sharing research data helps other researchers evaluate your findings, build on your work and to increase trust in your article. We encourage all our authors to make as much of their data publicly available as reasonably possible. Please note that your response to the following questions regarding the public data availability and the reasons for potentially not making data available will be available alongside your article upon publication. No. Has data associated with your study been deposited into a publicly available repository? Data will be made available on request.

## CRediT authorship contribution statement

**Xi Sun:** Writing – original draft, Software, Formal analysis. **Dexin Jia:** Validation, Data curation. **Yan Yu:** Supervision, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35001.

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