

RESEARCH ARTICLE OPEN ACCESS

The hsa_circ_0070354/miR-4322/RAB3B Axis Facilitates the Carcinogenesis and Development of Non-Small Cell Lung Cancer (NSCLC)

Yuejiao Huang¹  | Shiyi Qin² | Yu Zhang² | Xinliang Gu² | Ming Zheng² | Guojie Ling³ | Lei Yang³ | Shaoqing Ju² 

¹Department of Hematology and Oncology, Affiliated Hospital of Nantong University, Medical School of Nantong University, Nantong, Jiangsu, China | ²Department of Laboratory Medicine, Affiliated Hospital of Nantong University, Medical School of Nantong University, Nantong, Jiangsu, China | ³Department of Medical Oncology, Affiliated Tumor Hospital of Nantong University, Nantong, Jiangsu, China

Correspondence: Lei Yang (yanglei@ntu.edu.cn) | Shaoqing Ju (jsq814@hotmail.com)**Received:** 18 February 2025 | **Revised:** 24 April 2026 | **Accepted:** 15 May 2026**Keywords:** circular RNA | hsa_circ_0070354 | miR-4322 | NSCLC | RAB3B

ABSTRACT

Background: Circular RNA (hsa_circ_0070354), also known as circPTPN13, was demonstrated in our previous study that it can be used as a biomarker for diagnosing non-small cell lung cancer (NSCLC). However, the mechanism of its role in the occurrence and development of NSCLC has not been investigated.

Methods and Results: The cell functions of NSCLC cells are evaluated by transfecting with relevant mimics, inhibitors, or plasmids in cell lines. After knocking down hsa_circ_0070354, the expression of miR-4322 increased, and RAB3B decreased, and the expression changed inversely after overexpression of hsa_circ_0070354. Mechanically, hsa_circ_0070354 acts as a ceRNA to adsorb miR-4322 and modulate Ras-Related Protein Rab-3B (RAB3B) to facilitate the proliferation, migration, invasion, and EMT of NSCLC cells. MiR-4322 or RAB3B inhibition restored proliferation, migration, and invasion of NSCLC cells after silencing of hsa_circ_0070354. In addition, hsa_circ_0070354 knockdown suppressed tumor growth in vivo.

Conclusions: Collectively, our study reveals a promotive role of hsa_circ_0070354 in tumor proliferation, invasion, and migration by regulating the miR-4322/RAB3B axis and highlights that targeting the axis is a promising strategy for the intervention of NSCLC progression.

1 | Introduction

With the advancement of imaging technology and the increasing attention to physical examination, the diagnosis rate of early lung cancer has increased significantly. Although the incidence of breast cancer exceeds that of lung cancer, lung cancer remains the leading cause of cancer-related deaths globally,

with a five-year survival rate below 15% [1, 2]. This is due to the lack of typical and specific clinical manifestations of lung cancer. If diagnosed at an advanced stage, it will lose the opportunity for surgery; targeted therapy is not sensitive, which will lead to a poor prognosis for patients [3]. Non-small cell lung cancer (NSCLC) is the most common subtype of lung cancer, accounting for about 80%–85% of all lung cancer, and is the most

Abbreviations: (ceRNA), competitive endogenous RNA; (circRNA), circular RNA; (ECL), enhanced chemiluminescence; (EdU), 5-Ethynyl-2'-deoxyuridine; (EMT), epithelial-mesenchymal transition; (GAPDH), glyceraldehyde-3-phosphate dehydrogenase; (IHC), Hematoxylin-eosin (HE) staining and immunohistochemistry; (IRE1), Inositol-requiring enzyme 1; (miRNA), microRNA; (MRE), microRNA response elements; (MUT), wild-type (WT) or mutant; (NSCLC), non-small cell lung cancer; (OD), absorbance; (PI), phenylmethyl sulfonyl fluoride (PMSF) and phosphatase inhibitors; (PVDF), polyvinylidene difluoride; (qRT-PCR), quantitative real-time polymerase chain reaction; (RAB3B), Ras-Related Protein Rab-3B; (RIPA), radio-immunoprecipitation assay; (SDS-PAGE), sodium dodecyl sulfate-polyacrylamide gel electrophoresis; (shRNA), short hairpin RNA; (WB), western blot.

The first two authors contributed equally to this article.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2026 The Author(s). *Cancer Medicine* published by John Wiley & Sons Ltd.

difficult to treat [4]. With the progress of traditional radiotherapy, chemotherapy, and surgery, the rapid progress of targeted and immunotherapy is also the key to improving the survival time and prognosis of NSCLC [5]. However, in addition to the common mutations such as EGFR, ALK, and MET, the targets of molecular targeted therapy are still limited, which suggests that significant work remains in the search for new therapeutic targets.

Functional circular RNA (circRNA) has been found to effectively absorb microRNA (miRNA), thereby regulating downstream target genes after transcription, and can also serve as a repository or carrier of miRNA [6]. CircRNA can act as a competitive endogenous RNA (ceRNA) with multiple miRNA binding sites and can achieve mutual regulation by competitively binding to the common microRNA response elements (MRE) to play the role of miRNA sponge. This is the classic regulation model of circRNA, namely the triad regulation axis of circRNA/miRNA/mRNA [7]. In this new regulatory model, circRNA can competitively regulate the expression of multiple miRNAs with the same MER, while miRNA, in turn, can also regulate the post-transcriptional expression of multiple target genes. Moreover, multiple miRNAs can regulate the same target gene simultaneously [8, 9]. These multilayered interactions create a large and interconnected regulatory network. This regulatory model plays an important role in a variety of benign and malignant diseases, especially in the occurrence and development of tumors, including NSCLC [10, 11].

Existing studies have indicated that circRNA exhibits abnormal expression in NSCLC, which can be detected sensitively in the early stage. It can also predict and guide the disease progression according to its expression changes. CircRNA is an important tumor biomarker that is easy to detect [12, 13]. Furthermore, the expression of circRNA participates in the process of proliferation, invasion, and metastasis of NSCLC, which affects the adverse progression of the disease [14, 15]. Additionally, circRNA is also involved in regulating the sensitivity of NSCLC to chemotherapy and targeted drugs, thereby guiding the success or failure of treatment [16, 17]. Our previous research has confirmed that hsa_circ_0070354 has diagnostic efficacy as an NSCLC biomarker, but its role in the occurrence and development of NSCLC remains to be elucidated [18]. The research attempted to deeply investigate the function and regulatory mechanism of hsa_circ_0070354 in NSCLC, provide new strategies and targets for the diagnosis and treatment of NSCLC, and bring hope for the prognosis and survival of patients.

2 | Results

2.1 | Hsa_circ_0070354 Affects Cell Aggressive Progression in NSCLC Cells

To explore the functional role of hsa_circ_0070354 in NSCLC, we first knock down hsa_circ_0070354 expression in PC-9 and A549 cells, which have the highest hsa_circ_0070354 expression. Then, the expression of hsa_circ_0070354 was ectopically expressed in H1299 and H226 cells due to its lowest expression of hsa_circ_0070354. As expected, results showed that hsa_circ_0070354 successfully decreased about 50% changes

by shRNA-2 than controls, while OE-hsa_circ_0070354 could successfully up-regulate the expression by over 3-fold changes (Figure 1). Subsequently, cell growth, migration, and invasion in vitro assays were determined to evaluate the functions of hsa_circ_0070354 in NSCLC cells. Firstly, CCK-8, colony formation, and EdU experiments were conducted, and we found that silencing of hsa_circ_0070354 significantly inhibited PC-9 cell proliferation compared with the sh-NC group. On the contrary, ectopically expressed hsa_circ_0070354 increased cell viability in H1299 cells (Figure 1A–D). Moreover, relative to the control group, the migrated and invaded cells in transwell assays were remarkably reduced after the downregulation of hsa_circ_0070354 (Figure 1E–G Left). In contrast, the migratory and invasive capacities of H1299 and H226 cells were elevated after the overexpression of hsa_circ_0070354 (Figure 1E–G Right). Thus, these results revealed that hsa_circ_0070354 possessed an oncogenic function in NSCLC cells.

2.2 | Hsa_circ_0070354 Facilitates the Growth of NSCLC Cells in Vivo

Next, PC-9 cells with hsa_circ_0070354 stable knockdown or control cells were injected into the nude mice xenograft model to identify whether hsa_circ_0070354 could affect NSCLC cell tumorigenesis in vivo. Our findings revealed that silencing hsa_circ_0070354 obviously blocked tumor growth, and the tumors were smaller than those in the control group (Figure 2A). In the meantime, the tumor weight and volume in the sh-hsa_circ_0070354 group were also dramatically lower than those in the control group (Figure 2A,C). Similarly, in vivo experiments were performed using cells overexpressing circ_0070354, and the results revealed that upregulated circ_0070354 levels were sufficient to promote tumor growth, which was consistent with the findings from our knockdown studies (Figure 2B,C). Our findings indicate that hsa_circ_0070354 may play oncogenic roles in NSCLC progression in vivo. In addition, the IHC assays revealed that depression of hsa_circ_0070354 suppressed the expression of Ki-67 and Vimentin but promoted the expression of E-cadherin (Figure 2D). Taken together, our results suggest that the knockdown of hsa_circ_0070354 has anti-tumor activity in NSCLC in vivo.

2.3 | Hsa_circ_0070354 Sponged miR-4322 in NSCLC and Negatively Regulated Its Expression

According to the bioinformatics analysis conducted in the early stages by combining multiple online databases, potential targets of the ceRNA mechanism were selected [18]. Firstly, the expression changes of the pre-selected miRNAs were detected after upregulating or downregulating the expression of hsa_circ_0070354. It was demonstrated that only miR-1305 and miR-4322 were remarkably restrained by the overexpression of hsa_circ_0070354, while exerting the opposite effect by the down-regulation of hsa_circ_0070354 (Figure 3A). Next, we measured the expression of two miRNAs in NSCLC and normal bronchial epithelial cell lines by qRT-PCR. Results showed that miR-1305 and miR-4322 were lowly expressed in the majority of NSCLC cell lines compared to human normal bronchial epithelium cells (HBE) (Figure 3B). Similarly, miR-4322 was also

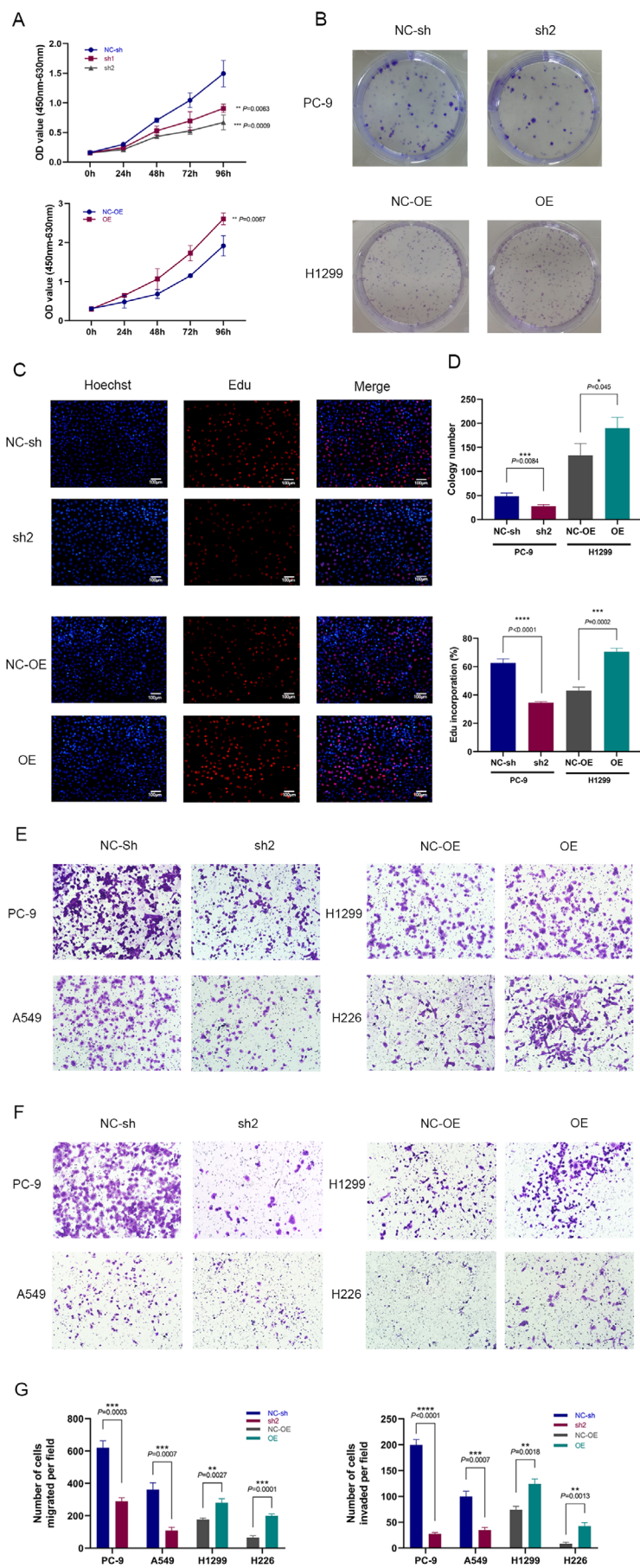


FIGURE 1 | Legend on next page.

FIGURE 1 | Hsa_circ_0070354 promotes NSCLC cell proliferation, migration, and invasion. (A) CCK-8 assays were employed to detect the cell viability of PC-9 and H1299 cells after overexpression or silencing transfection. (B) Colony formation assays were performed to detect the clonogenic ability of PC-9 and H1299 cells after transfection. (C) EdU analysis was adopted to detect cell proliferation of PC-9 and H1299 cells after transfection. Scale bar = 100 μ m. (D) Statistical graph of colony formation and EdU experiments. (E) Transwell assays were used to detect cell migration of NSCLC cells after transfection. (F) Transwell assays were used to detect cell invasion of NSCLC cells after transfection. Original magnification $\times$ 100. (G) Statistical graph of migration and invasion experiments.

generally lower in NSCLC tissues than in adjacent normal tissues, and its expression was opposite to that of hsa_circ_0070354 (Figure 3C,D). Afterward, the alignment of the potential binding site between hsa_circ_0070354 and miR-4322, as well as its mutant type, was constructed based on RNAhybrid prediction (Figure 3E). Further, the luciferase reporter vector containing wild-type or mutant putative binding sites of hsa_circ_0070354 was constructed. The results demonstrated that miR-4322 mimics could inhibit the luciferase activities of the wild-type hsa_circ_0070354 reporter vector, which could not be observed in the empty vector or mutant reporter vector (Figure 3F). It was also found that the luciferase signal of the wild-type hsa_circ_0070354 plasmid was decreased after the overexpression of miR-1305, but the statistical significance of the difference was poor. Therefore, accumulating evidence suggests that miR-4322 is a potential target of hsa_circ_0070354.

2.4 | Hsa_circ_0070354 Promoted NSCLC Progression by Sponging miR-4322

Since the function of miR-4322 in NSCLC and other tumors has not been reported, our study found that overexpression of miR-4322 presents a similar tumor inhibition effect as hsa_circ_0070354 silencing (Figure 4A–E). Conversely, inhibiting the expression of miR-4322 can promote the malignant process of NSCLC cells (Figure 4A–E). Previously, qRT-PCR analysis showed that the miR-4322 level was strikingly boosted after being transfected with miR-4322 mimics, while after being transfected with miR-4322 inhibitor, its expression was silenced (Figure 5A). To functionally confirm that hsa_circ_0070354 promotes NSCLC progression by sponging miR-4322, we transfected hsa_circ_0070354 OE and miR-4322 mimics into PC-9 and H1299 cells to examine whether the tumor-promoting effects of hsa_circ_0070354 overexpression could be reversed by miR-4322 upregulation. Indeed, we found that miR-4322 upregulation significantly suppressed the effects of hsa_circ_0070354 overexpression on proliferation (Figure 5B,C), migration (Figure 5D), and invasion (Figure 5E) of PC-9 cells. In addition, for the H1299 cell line, the knockdown of miR-4322 could partly rescue the tumor-repressing role of silenced hsa_circ_0070354 in proliferation, migration, and invasion (Figure 5B–E). The above results collectively demonstrated that hsa_circ_0070354 promoted NSCLC progression by sponging miR-4322.

2.5 | MiR-4322 Attenuates NSCLC Cell Growth, Metastasis, Invasion, and EMT by Targeting RAB3B

To further investigate the regulatory axis of hsa_circ_0070354/miR-4322, we utilized five online biological information databases (miRDB, miDIP, miPathDB, miRwalk, and TargetScan)

to predict a total of 85 potential target genes (Figure 6A). The screening criteria and parameters for each database, along with the 85-gene list, are now provided in Table S1. Combined with the Cytoscape analysis in our previous research [18], we obtained 10 potential functional genes (Figure 6B, Table S2). Among the differentially expressed genes in the TCGA database, RAB3B showed a consistent and statistically significant upregulation in both lung adenocarcinoma (Figure 6C) and lung squamous cell carcinoma (Figure 6D) tumor tissues compared to normal controls. Other genes, such as RSPO4 and PCDH17, also exhibited differential expression, but their expression patterns did not align with the predicted direction of regulation by miR-4322 or with the oncogenic phenotype observed in our functional assays. Therefore, RAB3B was prioritized for further validation.

Next, we asked whether hsa_circ_0070354's biological functions are dependent on its regulation of miR-4322 and RAB3B. Results from WB assays indicated that miR-4322 silencing augmented the expression level of RAB3B, while miR-4322 mimics reduced the mRNA and protein level of RAB3B (Figure 7A). Then, the transfection efficiency of RAB3B shRNA was checked, and two cell lines after transfection demonstrated a reduction in RAB3B expression (Figure 7B). WB results found that miR-4322 inhibition could enhance the expression of RAB3B, PCNA, N-cadherin, and vimentin, retard the expression of E-cadherin, insinuating that miR-4322 inhibition promoted the proliferation and metastasis of NSCLC cells. Moreover, transfection of RAB3B based on miR-4322 inhibition turned around the influence of miR-4322 inhibition on the protein expression above (Figure 7C). Based on RNAhybrid predictions, the wild-type and mutant sequences of the putative miR-4322 binding site within the RAB3B 3'UTR were designed and cloned into luciferase reporter vectors. The results demonstrated that miR-4322 mimics significantly suppressed the luciferase activity of the wild-type RAB3B reporter, whereas no such inhibitory effect was observed for the empty vector or the mutant reporter (Figure 7D). Additionally, for the part of rescue assays, CCK-8, clone forming, and transwell assays illustrated that transfected RAB3B shRNA could partly reverse the oncogenic functions caused by miR-4322 inhibition in A549 and H1299 cells (Figure 7E–H). In addition, the IHC assays revealed that depression of hsa_circ_0070354 suppressed the expression of RAB3B in vivo (Figure 7I). These observations indicated that miR-4322 attenuates NSCLC cell growth, metastasis, invasion, and EMT by targeting RAB3B.

3 | Discussion

CircRNAs are a class of endogenous non-coding RNAs with covalently closed loops, playing pivotal roles in carcinogenesis and cancer progression by sponging miRNAs, interacting with proteins, or encoding peptides [19, 20]. Growing research

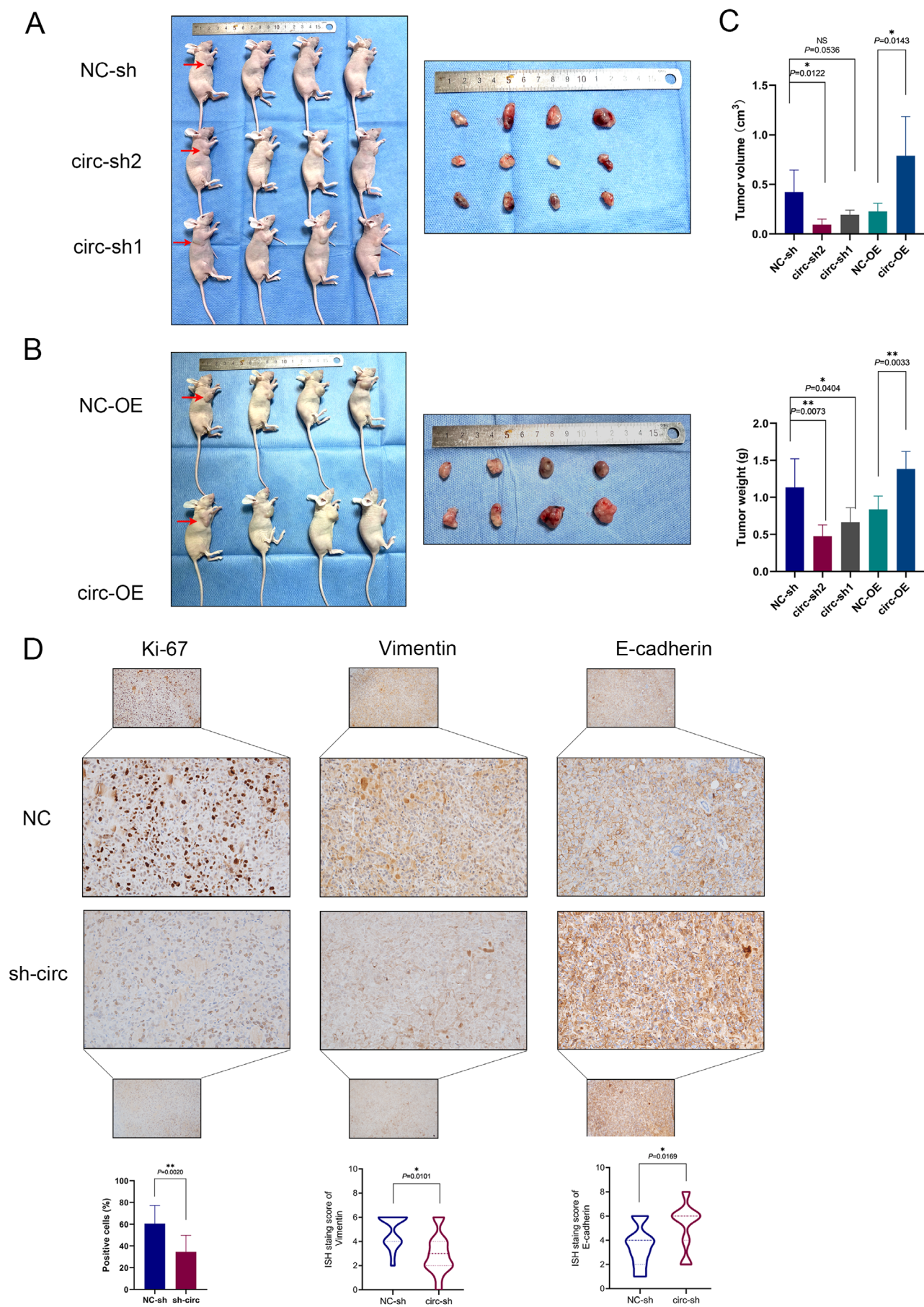


FIGURE 2 | Legend on next page.

FIGURE 2 | Hsa_circ_0070354 promotes tumor growth in vivo. (A) Hsa_circ_0070354 shRNA or the negative control stably transfected PC-9 cells were subcutaneously injected for in vivo tumorigenesis. (B) Hsa_circ_0070354 OE or the negative control stably transfected PC-9 cells were subcutaneously injected for in vivo tumorigenesis. (C) The volume and weight of subcutaneous xenograft tumors of PC-9 cells isolated from nude mice ($n = 4$ mice per group). (D) IHC staining for cell proliferation (Ki-67) and EMT markers (E-Cadherin and Vimentin) in subcutaneous xenograft tumors.

highlights their functions as transcriptional regulators, miRNA sponges, protein/peptide translation templates, as well as protein decoys, scaffolds, and recruiters [19]. Recent studies further clarify how circRNA can interact with DNA, RNA, and proteins to regulate biological processes, making circRNAs a focal point of cancer research [21]. The majority of circRNAs are primarily localized in the cytoplasm and act as miRNA sponges to regulate the expression of target miRNAs. Previous studies have examined differential expression of circRNAs between NSCLC and matched normal lung tissues through RNA sequencing or chip microarray [22]. Nonetheless, only a handful of circRNAs have been functionally identified. This study is based on previous analysis that hsa_circ_0070354 has a diagnostic marker for NSCLC to further investigate its functional role in the progression of NSCLC [18]. Building upon this clinical observation, the present study elucidates its functional oncogenic role and underlying molecular mechanism, thereby bridging the gap between its biomarker potential and biological significance in NSCLC pathogenesis.

It is noteworthy that circRNAs are generated through back-splicing of pre-mRNA and often exhibit expression patterns and functions that are distinct from their linear host genes [23, 24]. This functional decoupling is a recognized characteristic of many bona fide circRNAs [25]. Our proposed ceRNA mechanism for hsa_circ_0070354 operates in the cytoplasm via miRNA sequestration, a process that is mechanistically independent of nuclear transcription or the canonical mRNA function of its host gene, *PTPN13*. Therefore, while the impact on the host gene was not a focus of this study, the established paradigm of circRNA biology supports the conclusion that the observed oncogenic effects are likely attributable to the circular RNA transcript itself.

We first confirmed that hsa_circ_0070354 significantly promotes NSCLC cell proliferation, invasion, and metastasis both in vitro and in vivo. Consistent with its predominant cytoplasmic localization, we hypothesized it might function as a competitive endogenous RNA (ceRNA). Through integrated bioinformatics analysis and experimental validation, we identified miR-4322 as a direct target of hsa_circ_0070354. While miR-1305 was also predicted to bind hsa_circ_0070354, our dual-luciferase reporter assays demonstrated a significantly stronger and more specific interaction with miR-4322, leading us to focus on this less-characterized miRNA. While expression changes of miR-4322 have been observed in mesial temporal lobe epilepsy with hippocampal sclerosis, no statistically significant differences were found in diagnosis; furthermore, there is limited research on this microRNA's involvement in tumors and other diseases [26]. We found miR-4322 to be downregulated in NSCLC tissues and cells, and its restoration effectively counteracted the tumor-promoting effects of hsa_circ_0070354 overexpression. miRNAs can modulate various signaling pathways in tumor cells by controlling gene expression at the post-transcriptional level,

mainly through complementary pairing with the 3'-UTR of their target mRNAs, leading to mRNA degradation or translational inhibition [21, 27], _ENREF_27_ENREF_21. In this study, it was also screened and verified that miR-4322 regulates the gene expression of its target gene, RAB3B, and participates in the occurrence and development of NSCLC.

RAB3B, a small G protein, is a member of the Ras-related GTPase Rab family, which has the ability to regulate vesicle transport and participate in the formation and maintenance of the endoplasmic reticulum as well as intercellular signal transduction [28, 29]. A previous study demonstrated that knocking down RAB3B inhibits calcium-dependent exocytosis in rat anterior pituitary cells [30]. In addition to its presence in neuronal/secretory cells, RAB3B has also been identified in cancer cells [31, 32]. Tsunedomi et al. reported an upregulation of Rab3B in hepatocellular carcinoma, where it was deemed essential for acquiring tumor stem cell characteristics [31]. It played a pivotal role in extracellular vesicle secretion (such as exosomes) and potentially regulated the expression of related genes, making it a key gene for liver cancer treatment. In glioma, the expression of RAB3B was up-regulated, which inhibited cell apoptosis through cell cycle arrest and has prognostic significance [33]. Furthermore, RAB3B upregulation promoted the survival of prostate cancer cells [34]. It could also induce autophagy of tumor cells and promote cisplatin resistance of hypopharyngeal squamous cell carcinoma [35]. In acute myeloid leukemia, expression of RAB3B showed a positive correlation with the infiltration of M1 macrophages, and significant differences in sensitivity to 36 drugs were observed among high-risk groups, supporting RAB3B's inclusion as a biomarker in prognosis modeling [36]. In an article published concurrently with our study, it was found that RAB3B interacted with DDX6 and enhanced its stability, thereby endowing cancer cells with greater invasiveness [37]. It was consistent with our data on RAB3B in NSCLC. Despite these findings, the mechanism of RAB3B regulating the behavior of NSCLC cells is still unclear. RAB3B has been shown to exert its biological function by interacting with other proteins (e.g., binding to polyclonal immunoglobulin receptors to regulate epithelial cell transcytosis) [38–40]. Inositol-requiring enzyme 1 (IRE1) degrades pre-miR-3607 to regulate RAB3B expression at the mRNA and protein levels, thereby increasing the aggressiveness of luminal breast cancer cells [41]. RAB3B is also regulated by various miRNAs or circRNAs, such as circ_0000337, which upregulates RAB3B by secreting miR-155-5p to promote the proliferation of cervical cancer cells [42]. As a regulatory gene of miR-200b, inhibiting its expression could lead to a decrease in proliferation and invasion in breast cancer cells [32]. In gastric cancer, RAB3B was suggested to function as an oncogene that can potentially be regulated by the ceRNA mechanism, wherein lncRNA NEAT1 acts as a sponge to regulate RAB3B expression by sequestering miR-3158-5p [43]. In

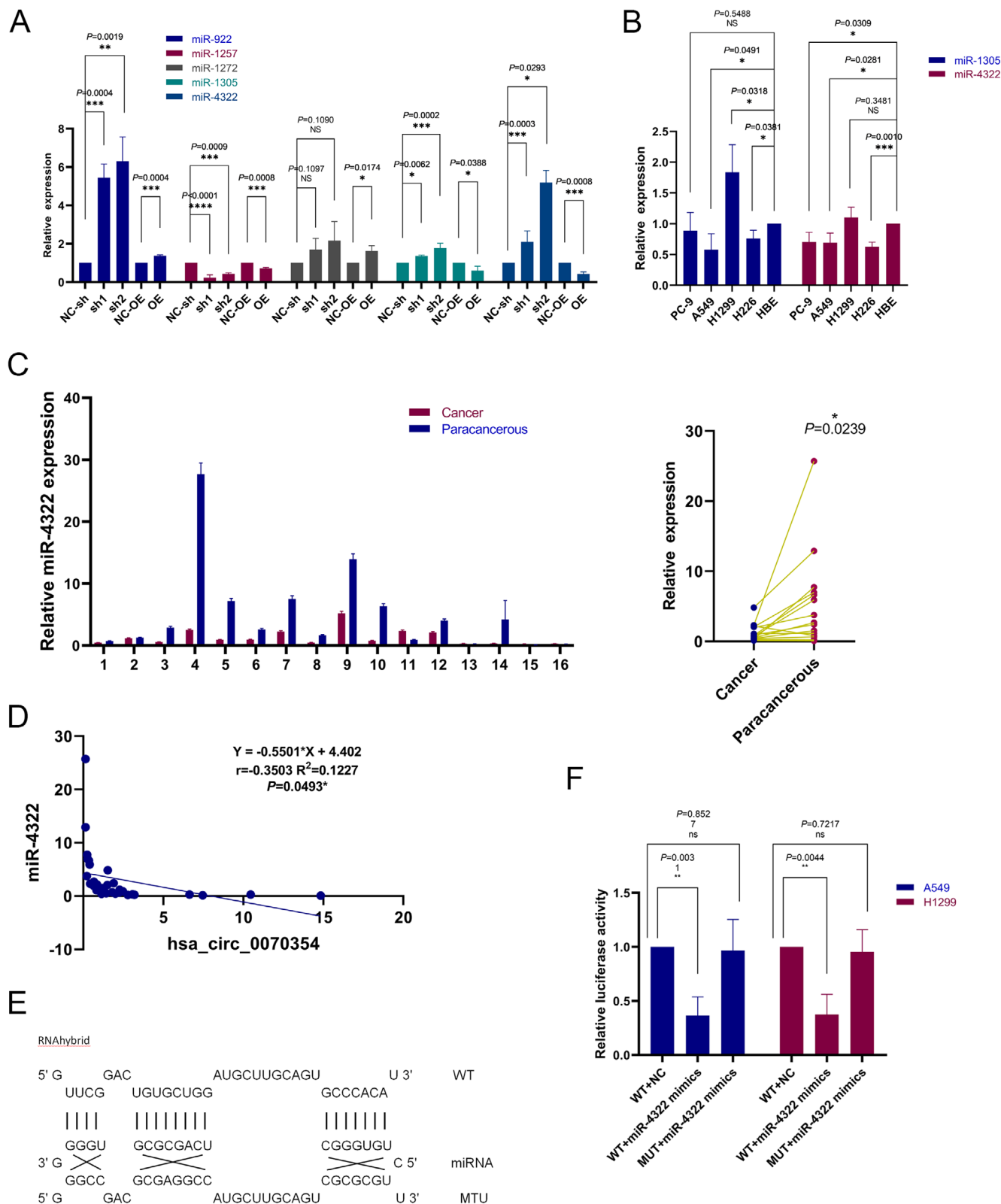


FIGURE 3 | Hsa_circ_0070354 negatively regulated the expression of miR-4322. (A) The qRT-PCR was administered to estimate the effects of hsa_circ_0070354 up-regulation or down-regulation on the expression of miRNAs. (B) The relative level of miR-4322 in NSCLC and normal bronchial epithelial cell lines was assayed through qRT-PCR. (C) The relative level of miR-4322 in 16 pairs of NSCLC and paracancerous tissues was assayed through qRT-PCR. (D) Correlation analysis of hsa_circ_0070354 and miR-4322 in NSCLC patients' tissues. (E) The seed sequences of hsa_circ_0070354 WT/Mut and miR-4322. (F) The interaction ability between hsa_circ_0070354 (wild-type (WT) and mutant (Mut)) and miR-4322 was evaluated by dual-luciferase reporter assay.

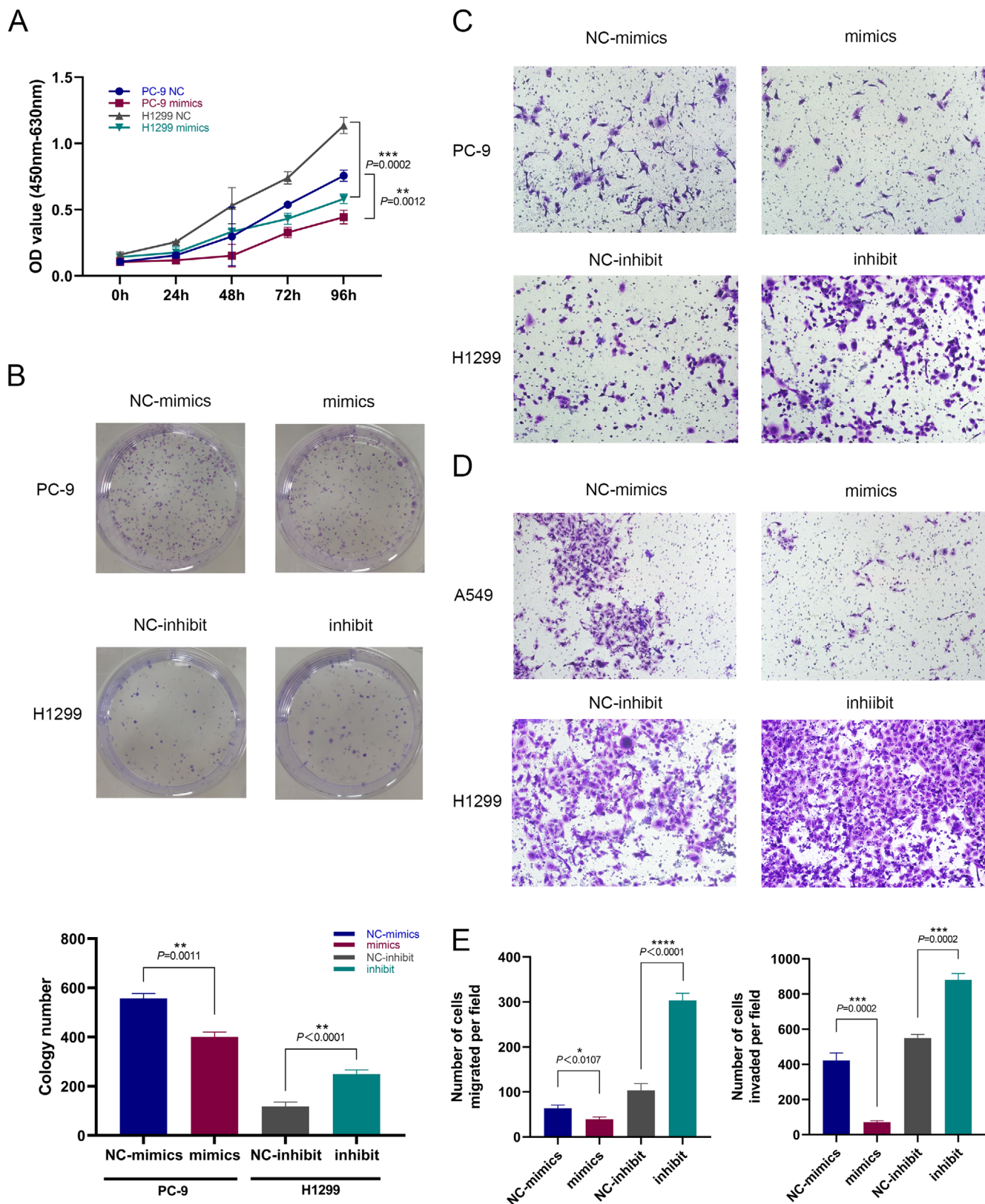


FIGURE 4 | MiR-4322 prevents NSCLC cell proliferation, migration, and invasion. (A) CCK-8 assays were employed to detect the cell viability after transfection. (B) Colony formation assays were performed to detect the clone's ability after transfection. (C) Transwell assays were used to detect cell migration of NSCLC cells after transfection. (D) Transwell assays were used to detect cell invasion of NSCLC cells after transfection. Original magnification $\times 100$. (E) Statistical graph of migration and invasion experiments.

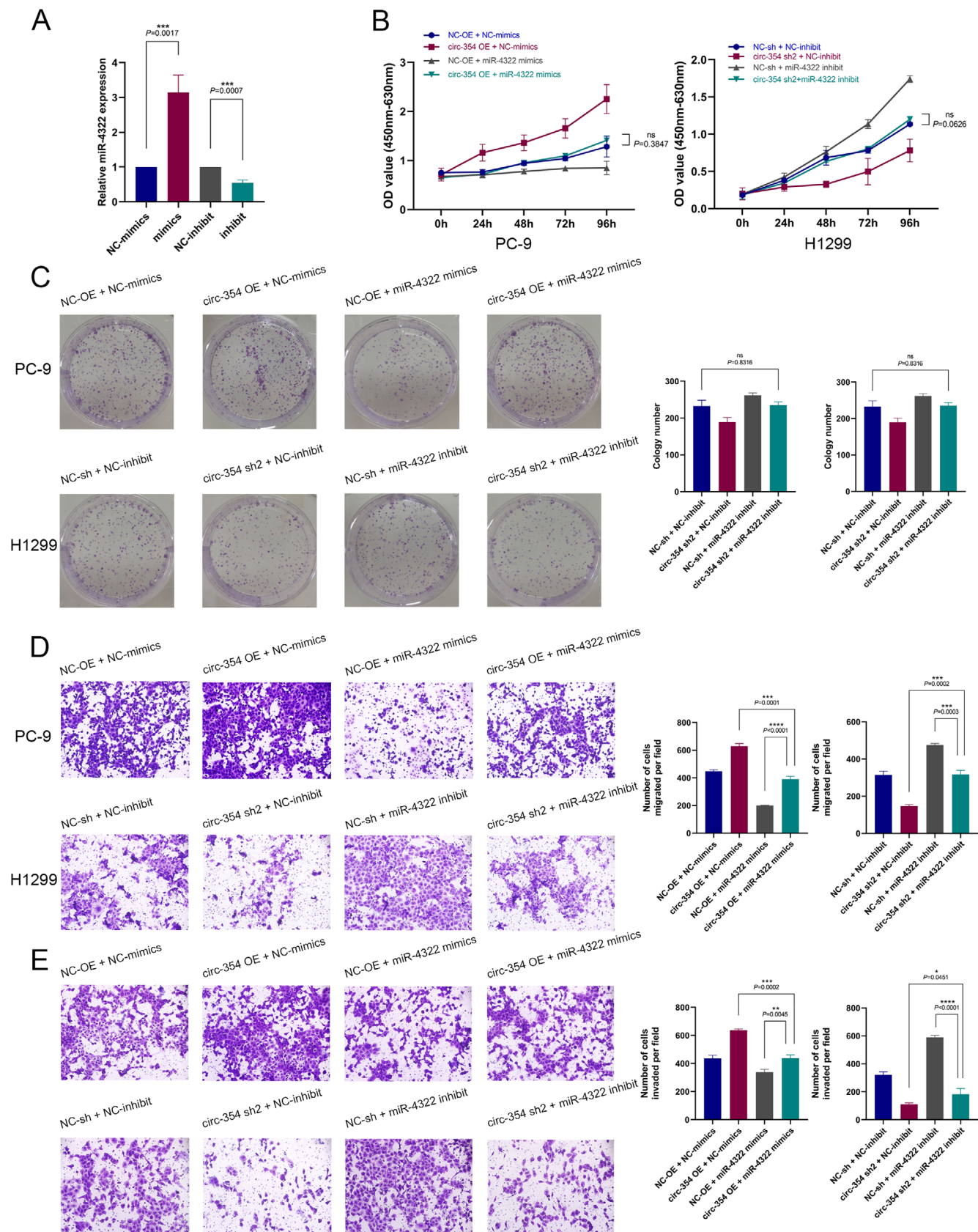


FIGURE 5 | The effect of hsa_circ_0070354 on NSCLC could be reversed by miR-4322. (A) Relative expression of miR-43,322 was measured by qRT-PCR after transfection of miR-4322 mimics or inhibitors. (B) CCK-8 assays were employed to detect the cell viability after co-transfection. (C) Colony formation assays were performed to detect the cloning ability after co-transfection. (D) Transwell assays were used to detect cell migration of NSCLC cells after co-transfection. (E) Transwell assays were used to detect cell invasion of NSCLC cells after co-transfection. Original magnification $\times 100$.

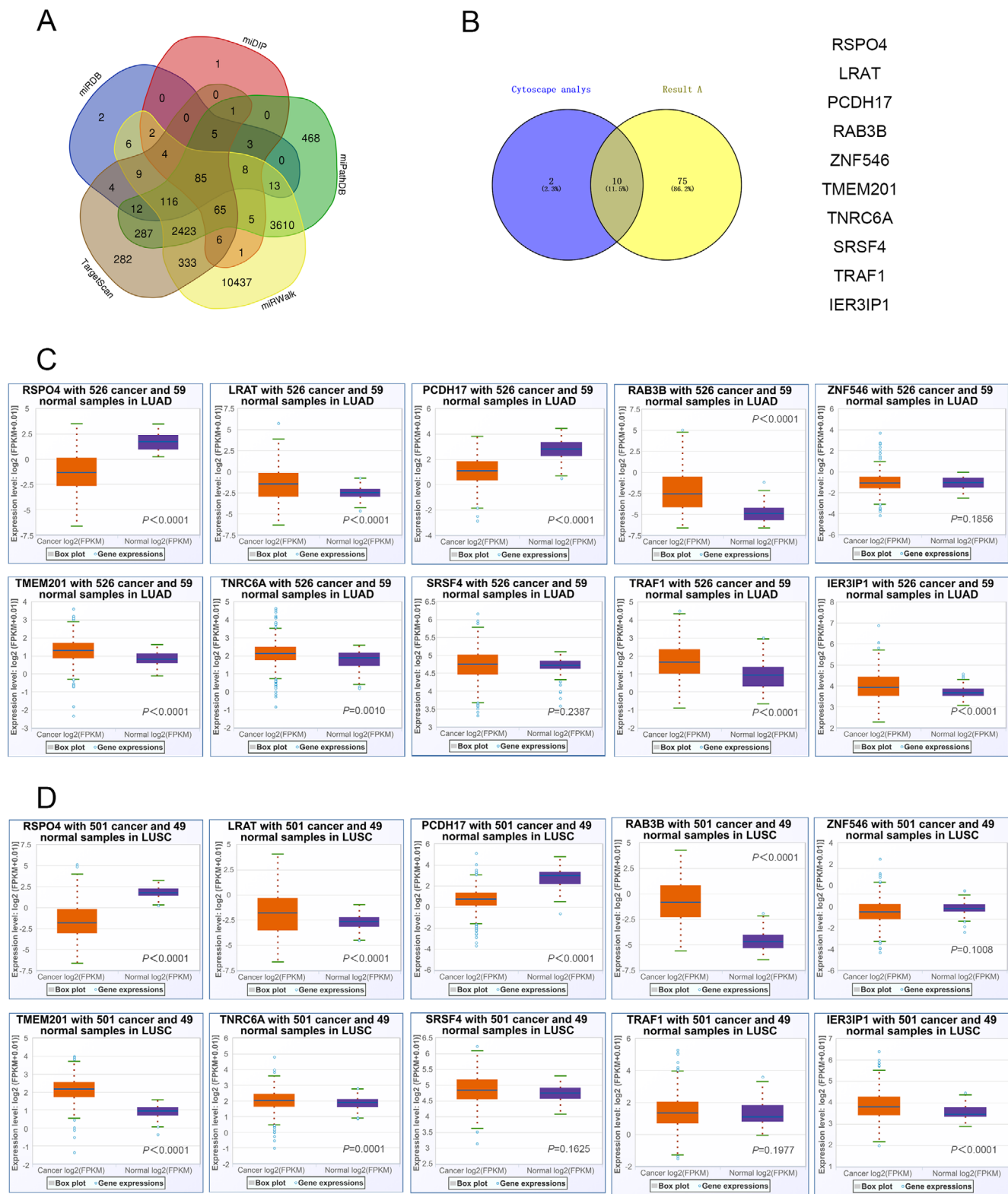


FIGURE 6 | Bioinformatics detection of target mRNAs of miR-4322. (A) The Bioinformatics online database was used to predict the potential target mRNAs that bind to miR-4322. (B) A Venn diagram evaluated the overlapped genes between five online databases and Cytoscape analysis predictions. (C) Prediction of 10 potential target mRNAs' expression in adenocarcinoma of the lung in the TCGA database by ENCORI analysis. (D) Prediction of 10 mRNAs' expression in squamous cell carcinoma of the lung in the TCGA database.

this study, we identified RAB3B as a direct and functional target of miR-4322. Importantly, the expression of RAB3B was negatively correlated with miR-4322 but positively correlated

with hsa_circ_0070354 in clinical samples. Collectively, these findings establish a novel ceRNA network—the hsa_circ_0070354/miR-4322/RAB3B axis—that contributes to

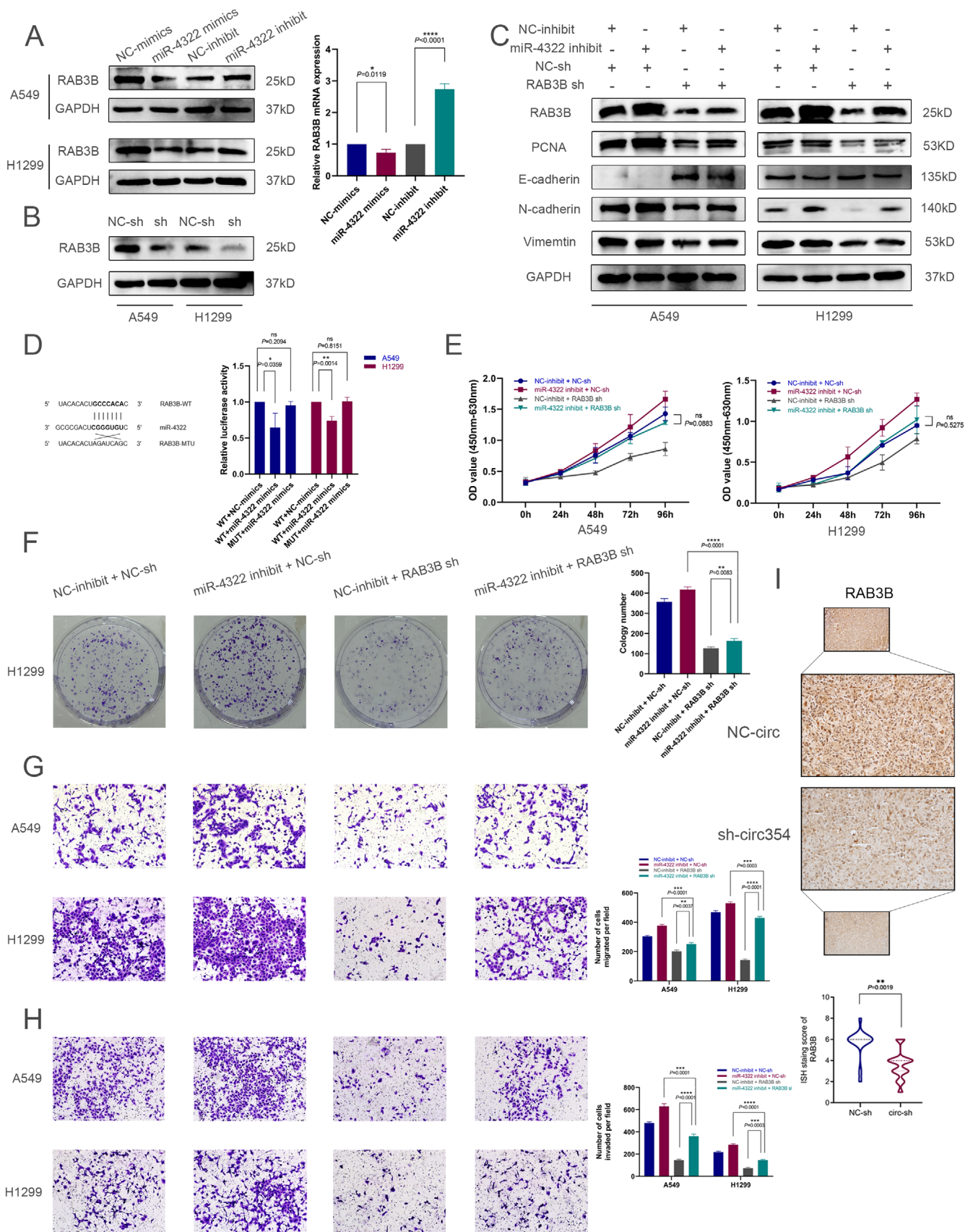


FIGURE 7 | Legend on next page.

FIGURE 7 | RAB3B acted as a molecular target of miR-4322. (A) The expression of RAB3B was measured by WB after transfection of miR-4322 mimics or inhibitors. (B) The interference efficiency of RAB3B was measured by WB after transfection of RAB3B shRNA. (C) The proliferation and metastasis-related proteins were determined using WB after co-transfection. (D) Diagrammatic sketch of the binding sites between miR-4322 and RAB3B in bioinformatics analysis (left). Dual-luciferase reporter assays were performed to detect the correlation between miR-4322 and RAB3B (right). (E) CCK-8 assays were employed to detect the cell viability after co-transfection. (F) Colony formation assays were performed to detect the clonogenic ability after co-transfection in H1299 cells. (G) Transwell assays were used to detect cell migration of NSCLC cells after co-transfection. (H) Transwell assays were used to detect cell invasion of NSCLC cells after co-transfection. Original magnification $\times 100$. (I) IHC staining for RAB3B in subcutaneous xenograft tumors.

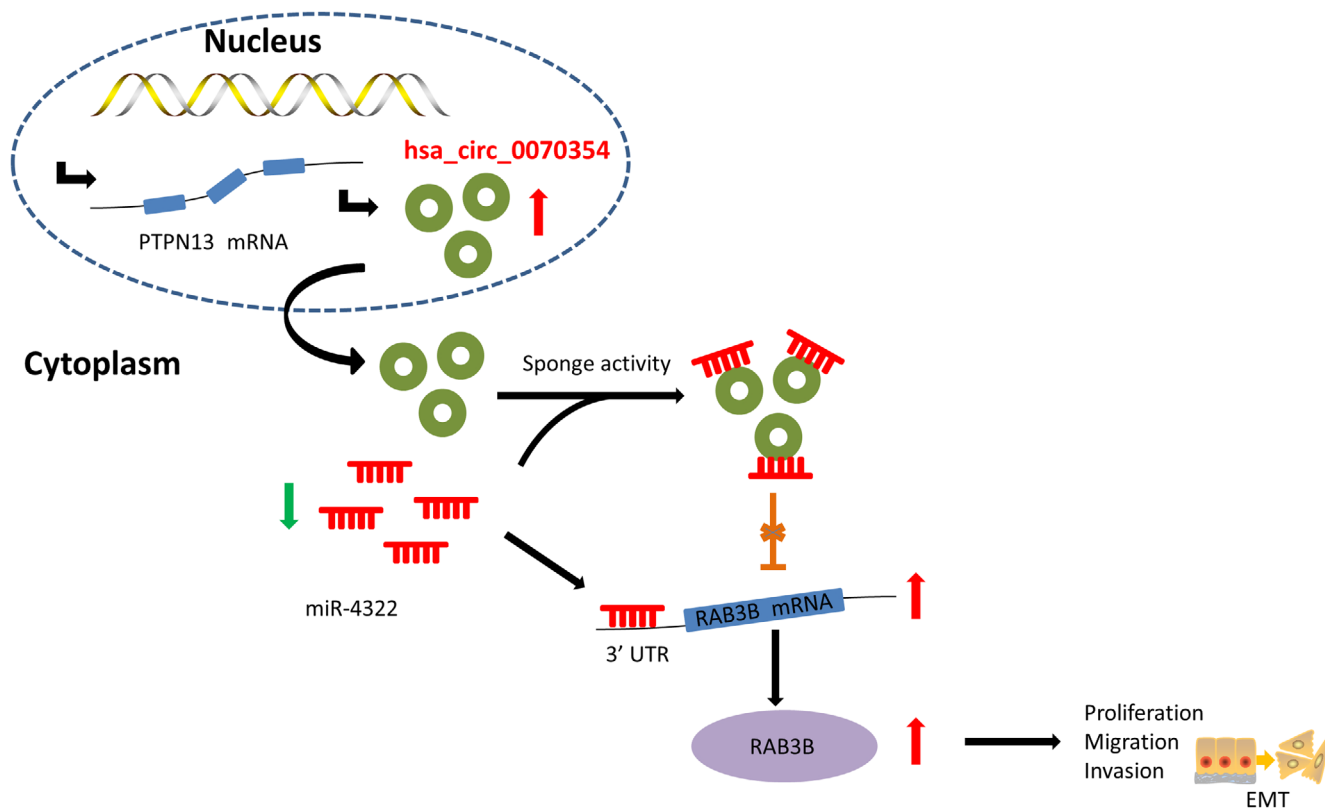


FIGURE 8 | Schematic diagram of the hsa_circ_0070354/miR-4322/RAB3B regulatory axis in NSCLC progression.

NSCLC progression (Figure 8). It was found for the first time that hsa_circ-0070354/miR-4322/RAB3B regulates the proliferation, invasion, and metastasis of NSCLC, which provides a theoretical basis for treatment target screening and prognosis analysis. Our subsequent experimental research will focus on conducting multiple rescue assays centered on circRNA-354, miR-4322, and RAB3B to validate our preliminary hypotheses. However, the signal pathways involved and the deeper mechanisms are still under further investigation.

The clinical relevance of this axis extends beyond mechanistic insight. First, it provides a plausible biological explanation for the elevated hsa_circ_0070354 levels we previously observed in NSCLC patients, linking a diagnostic biomarker to a pro-tumorigenic function. Evaluating the co-expression pattern of hsa_circ_0070354, miR-4322, and RAB3B in patient cohorts may enhance diagnostic precision or prognostic stratification compared to using hsa_circ_0070354 alone. Second, from a therapeutic perspective, this axis reveals new vulnerabilities. Targeting hsa_circ_0070354 with antisense

oligonucleotides (ASOs) or small interfering RNAs (siRNAs) could simultaneously inhibit RAB3B and de-repress miR-4322, achieving a dual antitumor effect. Although RAB3B's role in cancer is documented, our study is the first to delineate its upstream regulation by a circRNA-mediated sponge mechanism in NSCLC, offering a novel angle for intervention. The oncogenic function of the hsa_circ_0070354/miR-4322/RAB3B axis was consistently validated across multiple cell lines and in vivo models, underscoring its potential as a robust therapeutic target. Although we did not conduct the rescue experiments for hsa_circ_0070354 and RAB3B, it has been demonstrated in in vivo experiments that the reduction in hsa_circ_0070354 expression leads to a decrease in RAB3B expression (Figure 7I), thereby inhibiting tumor growth. Further verification through rescue experiments, both in vivo and in vitro, is warranted in the future.

In conclusion, our work advances the understanding of circRNA biology in NSCLC by functionally characterizing hsa_circ_0070354 and deciphering its role within a specific ceRNA

axis. The discovery of the hsa_circ_0070354/miR-4322/RAB3B regulatory network not only provides a mechanistic basis for the tumor-promoting effect of hsa_circ_0070354 but also highlights its potential value as a component of a multi-factor diagnostic panel and a candidate target for future RNA-based therapeutics. Future studies will explore the downstream signaling pathways governed by RAB3B in NSCLC and investigate the efficacy of targeting this axis in more complex preclinical models.

4 | Materials and Methods

4.1 | Cell Culture and Transfection

NSCLC cell lines (PC-9, A549, H1299, H226) and normal bronchial epithelial cells (HBE) were cultured as previously described [18]. Cell authentication and mycoplasma testing were performed prior to experiments. Transfection was conducted using Lipofectamine 3000 (Invitrogen, Cat. L3000015, Carlsbad, California, USA) at 50%–60% confluency. Specific shRNAs targeting hsa_circ_0070354 (sequence: 5'-CTTTCTGGGTAAGCCTAGCTG-3') and RAB3B (sequence: 5'-GGTGAAGACAGTCTACCGTCA-3'), along with a scrambled shRNA control (5'-GTTCTCCGAACGTGTCACGT-3'), as well as the hsa_circ_0070354 overexpression plasmid, were all synthesized and validated by GenePharma (Suzhou, China). miR-4322 mimic and inhibitor were obtained from Ribobio (Guangzhou, China).

4.2 | Tissue Samples

The human tissue samples were processed and analyzed according to protocols established in our prior study [18].

4.3 | Quantitative Real-Time PCR (qRT-PCR) Analysis

Total RNA extraction, cDNA synthesis, and qRT-PCR protocols followed established methods [18]. miR-4322 primers (Ribobio) and RAB3B primers (forward: 5'-TTCAAGGTGAAGACAGTCTACC-3'; reverse: 5'-GTAGGTCTTGATCTGAGTAGCC-3') were used. GAPDH and U6 served as normalization controls for mRNA/circRNA and miRNA, respectively.

4.4 | Cell Proliferation Assays

CCK-8 assay: The transfected NSCLC cells seeded in 96-well plates were incubated with CCK-8 reagent (Cat. CK04, Dojindo Laboratories, Kumamoto, Japan) for 2 h until the color turned orange. Absorbance at 450 nm was measured using a spectrophotometer (Bio-Tek, Vermont, USA). The cell viability (%) = $[\text{OD (Experiment)} - \text{OD (Blank)}] / [\text{OD (Control)} - \text{OD (Blank)}] \times 100\%$.

EdU assay: The transfected NSCLC cells treated with 50 μM EdU (Cat. C0075S, Beyotime, Shanghai, China) for 2 h were fixed, permeabilized, and stained with Hoechst 33342.

Fluorescence microscopy (Olympus, Tokyo, Japan) captured EdU-positive cells.

4.5 | Colony Formation Assay

Transfected cells (1×10^3 /well) were cultured for 10–14 days with the medium replaced every 4 days, fixed with paraformaldehyde, and stained with crystal violet (Cat. C0121, Beyotime, Shanghai, China). For counting the colony-forming units, the cell colonies were dried in air and photographed.

4.6 | Migration and Invasion Assay

For transwell migration and invasion assays, cells were pre-treated with 1 $\mu\text{g/mL}$ Mitomycin C (Cat. M5353, Sigma-Aldrich, Darmstadt, Germany) for 1 h to inhibit proliferation prior to being seeded into the inserts. Both invasion and migration assays were conducted using transwell chambers (Corning, New York, USA), with the difference being that in the invasion assay, 100 μL of Matrigel (Cat. 354234, Corning, New York, USA) was pre-spread at a 1:8 dilution in the upper chamber and cultured overnight to allow it to gel. Then, the transfected cells (1×10^5 /well) in serum-free medium were added to the upper chamber, while 20% FBS medium filled the lower chamber. After incubating with 5% CO_2 for 24–36 h, the cells in the upper membrane surfaces were carefully removed by cotton swabs, while the transwell chambers were fixed in 4% paraformaldehyde solution and stained using 0.1% crystal violet for 20 min. Finally, the number of migrated or invaded cells was photographed and counted in five representative fields under an inverted microscope (Olympus, Tokyo, Japan). For each experimental condition, transwell assays were performed in three independent replicates. Migrated or invaded cells were counted in five randomly selected fields per insert using ImageJ software (National Institutes of Health, USA), and the results from all replicates were pooled for statistical analysis.

4.7 | Dual-Luciferase Reporter Assay

The hsa_circ_0070354 and RAB3B sequences containing wild-type (WT) or mutant (MUT) miR-4322 binding sites were purchased from Genepharma (Suzhou, China). The WT and MUT plasmids were cotransfected into NSCLC cells with miR-4322/1305 mimics, or NC mimics using Lipofectamine 3000 (Cat. L3000015, Invitrogen, California, USA). Luciferase activity was measured 48 h post-transfection using a Dual-Luciferase Kit (Cat. E2920, Promega, Wisconsin, USA), normalized to Renilla activity.

4.8 | Xenograft Model

BALB/c nude mice (4–5 weeks old) were bred and experimented upon at the animal center of the Medical School in Nantong University, and the procedures were conducted in accordance with the Nantong University ethical guidelines. Briefly, 1×10^6 transfected PC-9 cells were subcutaneously injected into the left armpit after being diluted with PBS and Matrigel to 100 μL .

After 30 days, all the mice were euthanized, and then the tumors were separated and weighed. Tumor volume (calculated as $[\text{length} \times \text{width}^2]/2$) was recorded.

4.9 | Hematoxylin–Eosin (HE) Staining and Immunohistochemistry (IHC)

The preparation and staining of tissue sections are assisted by the automatic dehydrator, embedding machine, staining and mounting machine of the pathology department in Third People's Hospital of Nantong University, which are used to automate the process. In brief, the tumor tissues were first obtained, fixed in 10% formalin, dehydrated in graded alcohol, embedded in paraffin, sliced, and stained. In IHC, however, antigen retrieval, blocking, and incubation with the primary antibody and secondary antibody are also required. The following primary antibodies are used: RAB3B (1:100, Cat. sc-81,911, Santa Cruz, California, USA), Ki-67 (1:100, Cat. sc-23,900, Santa Cruz, California, USA), E-cadherin (1:200, Cat. 3195S, Cell Signaling Technology, Massachusetts, USA), and Vimentin (1:200, Cat. 5741S, Cell Signaling Technology, Massachusetts, USA). The staining intensity was scored as follows: 0 (no staining), 1 (weak), 2 (moderate), and 3 (strong). The staining extent was scored as follows: 0 (0%), 1 (1%–25%), 2 (26%–50%), 3 (51%–75%), and 4 (76%–100%). The final expression score was calculated by multiplying the intensity score by the extent score, yielding a range from 0 to 12 [11].

4.10 | Western Blotting (WB)

First, cells or tissues were extracted using a radio-immunoprecipitation assay (RIPA) lysis buffer (Cat. R0010, Solarbio, Beijing, China) containing phenylmethyl sulfonyl fluoride (PMSF) and phosphatase inhibitors (PI), and the protein concentration was determined. The protein loading amount was adjusted based on the protein concentration and the expression of the control GAPDH (1:1000, Cat. AC002, ABclonal, Wuhan, China). Proteins were separated by 10% or 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under initial conditions of 80 V followed by voltage conditions of 100–120 V, and transferred to a polyvinylidene difluoride (PVDF) membrane (Cat. IPFL00010, Millipore, Massachusetts, USA) at 250 mA. Subsequently, the membrane is blocked with TBST containing 5% fat-free dried milk at room temperature for 2 h, incubated with primary antibody (1:1000, same as above) at 4°C overnight, and incubated with the horseradish peroxidase (HRP)-conjugated secondary antibody (1:3000, Cat. FMS-MS01, Nanjing, China and Cat. 7074S, Cell Signaling Technology, Massachusetts, USA) for 90 min at room temperature. An enhanced chemiluminescence (ECL) solution (New Cell & Molecular Biotech, Cat. P10060, Suzhou, China) is prepared according to the instructions (1:1), added onto the PVDF membrane, and incubated in darkness for 1–5 min at room temperature before imaging using a multifunctional imaging system (BIO-RAD, California, USA).

4.11 | Statistical Analysis

Data from triplicate experiments were presented as the means $\pm$ standard deviation (SD). SPSS 19.0 (SPSS Inc., Chicago, USA)

and GraphPad Prism 8.0 (GraphPad Software Inc., California, USA) were used for statistical analyses and figures. Differences between the two groups were tested using unpaired Student's *t*-tests, while one-way ANOVA was used to compare three or more groups. Linear correlation analysis was calculated to assess the correlations between hsa_circ_0070354, miR-4322, and RAB3B mRNA expression. $p < 0.05$ was considered statistically significant ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and $***p < 0.001$).

Author Contributions

The research framework was collaboratively designed by YJH and SQJ. SQJ and L.Y. provided methodological oversight, intellectual input, and substantive editorial amendments. L.Y. contributed clinical expertise and coordinated the acquisition of NSCLC patient datasets. Experimental investigations were conducted by SYQ, Y.Z., and M.Z. under supervisory guidance. XLG and SYQ managed information systematization and executed analytical procedures. The primary manuscript composition and visual documentation were completed by YJH. All collaborators participated in scholarly discussions, reviewed the final content, and endorsed its publication.

Acknowledgements

The authors have nothing to report.

Funding

This project was supported by grants from the National Natural Science Foundation of China (Nos. 82272411, 82072363), the Science and Technology Project of Jiangsu Province (BE2023741), and the Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX24_3582).

Ethics Statement

Human subject investigations received formal approval from the Affiliated Hospital of Nantong University's Institutional Review Board (Approval ID: 2018-L055), with documented participant consent obtained through written agreements. The above animal study was performed following the Basel Declaration and the approval of the Animal Use and Care Committee of Nantong University (Protocol ID: S20221222-006).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. R. L. Siegel, K. D. Miller, H. E. Fuchs, and A. Jemal, "Cancer Statistics," *A Cancer Journal for Clinicians* 72 (2022): 7–33, <https://doi.org/10.3322/caac.21708>.
2. Z. Megyesfalvi, C. M. Gay, H. Popper, et al., "Clinical Insights Into Small Cell Lung Cancer: Tumor Heterogeneity, Diagnosis, Therapy, and Future Directions," *A Cancer Journal for Clinicians* 73 (2023): 620–652, <https://doi.org/10.3322/caac.21785>.
3. H. Mithoowani and M. Febbraro, "Non-Small-Cell Lung Cancer in 2022: A Review for General Practitioners in Oncology," *Current Oncology* 29 (2022): 1828–1839, <https://doi.org/10.3390/curroncol29030150>.

4. C. Slatore, S. C. Lareau, and B. Fahy, "Staging of Lung Cancer," *American Journal of Respiratory and Critical Care Medicine* 205 (2022): P17–P19, <https://doi.org/10.1016/j.rccm.2059P17>.
5. P. Chen, Y. Liu, Y. Wen, and C. Zhou, "Non-Small Cell Lung Cancer in China," *Cancer Communications (London, England)* 42 (2022): 937–970, <https://doi.org/10.1002/cac2.12359>.
6. Q. Liu and S. Li, "Exosomal circRNAs: Novel Biomarkers and Therapeutic Targets for Urinary Tumors," *Cancer Letters* 588 (2024): 216759, <https://doi.org/10.1016/j.canlet.2024.216759>.
7. M. Abbaszadeh, M. Karimi, and S. Rajaei, "The Landscape of Non-Coding RNAs in the Immunopathogenesis of Endometriosis," *Frontiers in Immunology* 14 (2023): 1223828, <https://doi.org/10.3389/fimmu.2023.1223828>.
8. K. Kielbowski, K. Ptaszynski, J. Wojcik, et al., "The Role of Selected Non-Coding RNAs in the Biology of Non-Small Cell Lung Cancer," *Advanced Medical Science* 68 (2023): 121–137, <https://doi.org/10.1016/j.advms.2023.02.004>.
9. M. Alahdal and E. Elkord, "Non-Coding RNAs in Cancer Immunotherapy: Predictive Biomarkers and Targets," *Clinical and Translational Medicine* 13 (2023): e1425, <https://doi.org/10.1002/ctm2.1425>.
10. X. Chen, J. Gu, J. Huang, et al., "Characterization of circRNAs in Established Osimertinib-Resistant Non-Small Cell Lung Cancer Cell Lines," *International Journal of Molecular Medicine* 52, no. 5 (2023): 102, <https://doi.org/10.3892/ijmm.2023.5305>.
11. Y. F. Tang, Z. H. Liu, L. Y. Zhang, et al., "circ_PPAPDC1A Promotes Osimertinib Resistance by Sponging the miR-30a-3p/IGF1R Pathway in Non-Small Cell Lung Cancer (NSCLC)," *Molecular Cancer* 23 (2024): 91, <https://doi.org/10.1186/s12943-024-01998-w>.
12. Z. Chen, X. Ma, W. Chen, et al., "Exosome-Transported circ_0061407 and circ_0008103 Play a Tumour-Repressive Role and Show Diagnostic Value in Non-Small-Cell Lung Cancer," *Journal of Translational Medicine* 22 (2024): 427, <https://doi.org/10.1186/s12967-024-05215-6>.
13. X. Fan, Q. Zhang, S. Qin, et al., "CircBRIP1: A Plasma Diagnostic Marker for Non-Small-Cell Lung Cancer," *Journal of Cancer Research and Clinical Oncology* 150 (2024): 83, <https://doi.org/10.1007/s00432-023-05558-5>.
14. S. Kahkesh, S. M. Khoshnazar, Y. Gholinezhad, et al., "The Potential Role of Circular RNAs -Regulated PI3K Signaling in Non-Small Cell Lung Cancer: Molecular Insights and Clinical Perspective," *Pathology, Research and Practice* 257 (2024): 155316, <https://doi.org/10.1016/j.prp.2024.155316>.
15. T. Chen, Z. Li, J. Chen, and Z. Xu, "Circ_0000877 Accelerates Proliferation and Immune Escape of Non-Small Cell Lung Cancer Cells by Regulating microRNA-637/E2F2 Axis," *Environmental Toxicology* 39 (2024): 2980–2992, <https://doi.org/10.1002/tox.24172>.
16. E. Gong, J. Pan, Z. Ye, et al., "Ganoderic Acid A Suppresses Autophagy by Regulating the circFLNA/miR-486-3p/CYP1A1/XRCC1 Axis to Strengthen the Sensitivity of Lung Cancer Cells to Cisplatin," *Journal of Pharmacy and Pharmacology* 76 (2024): 354–367, <https://doi.org/10.1093/jpp/rgad116>.
17. H. He and T. Li, "Hsa_circ_0000190 Promotes NSCLC Cell Resistance to Cisplatin via the Modulation of the miR-1253/IL-6 Axis," *Analytical Cellular Pathology (Amsterdam)* 2024 (2024): 6647810, <https://doi.org/10.1155/2024/6647810>.
18. Y. Huang, S. Qin, X. Gu, et al., "Comprehensive Assessment of Serum hsa_circ_0070354 as a Novel Diagnostic and Predictive Biomarker in Non-Small Cell Lung Cancer," *Frontiers in Genetics* 12 (2021): 796776, <https://doi.org/10.3389/fgene.2021.796776>.
19. B. Liu, H. Ma, X. Liu, and W. Xing, "CircSCN8A Suppresses Malignant Progression and Induces Ferroptosis in Non-Small Cell Lung Cancer by Regulating miR-1290/ACSL4 Axis," *Cell Cycle* 22 (2023): 758–776, <https://doi.org/10.1080/15384101.2022.2154543>.
20. H. H. Chen, T. N. Zhang, Q. J. Wu, X. M. Huang, and Y. H. Zhao, "Circular RNAs in Lung Cancer: Recent Advances and Future Perspectives," *Frontiers in Oncology* 11 (2021): 664290, <https://doi.org/10.3389/fonc.2021.664290>.
21. Y. Zhang, S. Zeng, and T. Wang, "Circular RNA hsa_circ_0002360 Promotes Non-Small Cell Lung Cancer Progression Through Upregulating Matrix Metalloproteinase 16 and Sponging Multiple miRNAs," *Bioengineered* 12 (2021): 12767–12777, <https://doi.org/10.1080/21655979.2021.1999370>.
22. C. Yu, Z. Cheng, S. Cui, et al., "circFOXO1 Promotes Proliferation of Non-Small Cell Lung Carcinoma Cells by Acting as a ceRNA to Upregulate FAM83D," *Journal of Experimental & Clinical Cancer Research* 39 (2020): 55, <https://doi.org/10.1186/s13046-020-01555-5>.
23. S. Memczak, M. Jens, A. Elefsinioti, et al., "Circular RNAs Are a Large Class of Animal RNAs With Regulatory Potency," *Nature* 495 (2013): 333–338, <https://doi.org/10.1038/nature11928>.
24. T. B. Hansen, T. I. Jensen, B. H. Clausen, et al., "Natural RNA Circles Function as Efficient microRNA Sponges," *Nature* 495 (2013): 384–388, <https://doi.org/10.1038/nature11993>.
25. Q. Zheng, C. Bao, W. Guo, et al., "Circular RNA Profiling Reveals an Abundant circHIPK3 That Regulates Cell Growth by Sponging Multiple miRNAs," *Nature Communications* 7 (2016): 11215, <https://doi.org/10.1038/ncomms11215>.
26. S. Yan, H. Zhang, W. Xie, et al., "Altered microRNA Profiles in Plasma Exosomes From Mesial Temporal Lobe Epilepsy With Hippocampal Sclerosis," *Oncotarget* 8 (2017): 4136–4146, <https://doi.org/10.18632/oncotarget.13744>.
27. G. Liu, H. Shi, L. Deng, et al., "Circular RNA Circ-FOXO1 Facilitates Cell Progression as ceRNA to Target PDPF and MACC1 by Sponging miR-1304-5p in Non-Small Cell Lung Cancer," *Biochemical and Biophysical Research Communications* 513 (2019): 207–212, <https://doi.org/10.1016/j.bbrc.2019.03.213>.
28. T. Tsetsenis, T. J. Younts, C. Q. Chiu, P. S. Kaeser, P. E. Castillo, and T. C. Südhof, "Rab3B Protein Is Required for Long-Term Depression of Hippocampal Inhibitory Synapses and for Normal Reversal Learning," *Proceedings of the National Academy of Sciences of the United States of America* 108 (2011): 14300–14305, <https://doi.org/10.1073/pnas.1112237108>.
29. F. Rotondo, B. W. Scheithauer, K. Kovacs, and D. C. Bell, "Rab3B Immunoreactivity in Human Pituitary Adenomas," *Applied Immunohistochemistry & Molecular Morphology* 17 (2009): 185–188, <https://doi.org/10.1097/PAI.0b013e31818fa0ed>.
30. P. M. Lledo, P. Vernier, J. D. Vincent, W. T. Mason, and R. Zorec, "Inhibition of Rab3B Expression Attenuates ca(2+)-dependent Exocytosis in Rat Anterior Pituitary Cells," *Nature* 364 (1993): 540–544, <https://doi.org/10.1038/364540a0>.
31. R. Tsunedomi, K. Yoshimura, Y. Kimura, et al., "Elevated Expression of RAB3B Plays Important Roles in Chemoresistance and Metastatic Potential of Hepatoma Cells," *BMC Cancer* 22 (2022): 260, <https://doi.org/10.1186/s12885-022-09370-1>.
32. Q. Liu, H. Tang, X. Liu, et al., "miR-200b as a Prognostic Factor Targets Multiple Members of RAB Family in Glioma," *Medical Oncology* 31 (2014): 859, <https://doi.org/10.1007/s12032-014-0859-x>.
33. Q. Luo, Y. Liu, Z. Yuan, L. Huang, and B. Diao, "Expression of Rab3b in Human Glioma: Influence on Cell Proliferation and Apoptosis," *Current Pharmaceutical Design* 27 (2021): 989–995, <https://doi.org/10.2174/1381612826666200917145228>.
34. P. Y. Tan, C. W. Chang, K. R. Chng, K. D. S. A. Wansa, W. K. Sung, and E. Cheung, "Integration of Regulatory Networks by NKX3-1 Promotes Androgen-Dependent Prostate Cancer Survival," *Molecular and Cellular Biology* 32 (2012): 399–414, <https://doi.org/10.1128/MCB.05958-11>.

35. C. Lin, Y. Chen, F. Zhang, et al., "Encoding Gene RAB3B Exists in Linear Chromosomal and Circular Extrachromosomal DNA and Contributes to Cisplatin Resistance of Hypopharyngeal Squamous Cell Carcinoma via Inducing Autophagy," *Cell Death & Disease* 13 (2022): 171, <https://doi.org/10.1038/s41419-022-04627-w>.
36. C. Liu and L. Liu, "Identification and Immunoassay of Prognostic Genes Associated With the Complement System in Acute Myeloid Leukemia," *Journal of the Formosan Medical Association* 123, no. 8 (2024): 904–915, <https://doi.org/10.1016/j.jfma.2024.01.024>.
37. G. Yao, S. Yu, F. Hou, et al., "Rab3B Enhances the Stabilization of DDX6 to Promote Lung Adenocarcinoma Aggressiveness," *Molecular Medicine* 30 (2024): 75, <https://doi.org/10.1186/s10020-024-00848-1>.
38. S. C. D. van IJzendoorn, M. J. Tuvim, T. Weimbs, et al., "Direct Interaction Between Rab3b and the Polymeric Immunoglobulin Receptor Controls Ligand-Stimulated Transcytosis in Epithelial Cells," *Developmental Cell* 2 (2002): 219–228, [https://doi.org/10.1016/s1534-5807\(02\)00115-6](https://doi.org/10.1016/s1534-5807(02)00115-6).
39. N. Nishimura, K. Araki, W. Shinahara, et al., "Interaction of Rab3B With Microtubule-Binding Protein Gas8 in NIH 3T3 Cells," *Archives of Biochemistry and Biophysics* 474 (2008): 136–142, <https://doi.org/10.1016/j.abb.2008.03.0327>.
40. J. Hu, Q. Wang, X. Fan, et al., "Long Noncoding RNA ENST00000436340 Promotes Podocyte Injury in Diabetic Kidney Disease by Facilitating the Association of PTBP1 With RAB3B," *Cell Death & Disease* 14 (2023): 130, <https://doi.org/10.1038/s41419-023-05658-7>.
41. K. Zhang, H. Liu, Z. Song, et al., "The UPR Transducer IRE1 Promotes Breast Cancer Malignancy by Degrading Tumor Suppressor microRNAs," *iScience* 23 (2020): 101503, <https://doi.org/10.1016/j.isci.2020.101503>.
42. J. Xu, B. Xue, M. Gong, et al., "circ_0000337 Promotes the Progression of Cervical Cancer by miR-155-5p/RAB3B Axis," *Biochemical Genetics* 62 (2024): 2195–2209, <https://doi.org/10.1007/s10528-023-10534-2>.
43. C. Li, X. Zou, Q. Cai, et al., "Comprehensive Expression Profile Analysis of Neutrophil Extracellular Trap-Affected Genes in Gastric Cancer Cells and the Clinical Significance of lncRNA NEAT1-Related Signaling," *Frontiers in Oncology* 12 (2022): 798531, <https://doi.org/10.3389/fonc.2022.798531>.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** The transfection efficiency of hsa_circ_0070354 in NSCLC cell lines, transfected with siRNAs, full-length or control vectors, was examined by qRT-PCR. **Table S1:** The detailed in silico prediction data corresponding to Figure 6A. **Table S2:** The detailed in silico prediction data corresponding to Figure 6B.