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# Circ0021205 aggravates the progression of non-small cell lung cancer by targeting miRNA-16-5p/VEGFA

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**Abstract. – OBJECTIVE:** To uncover the role of circ0021205 in the progression of non-small cell lung cancer (NSCLC) and its molecular mechanism.

**PATIENTS AND METHODS:** The expression level of circ0021205 in NSCLC tissues and cells was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The relationship between circ0021205 level and the pathological indexes of NSCLC was analyzed by the Chi-square test. The Kaplan-Meier curves were depicted for evaluating the influe[illegible] circ0021205 on the survival of NSCLC p[illegible]t. After altering circ0021205 level in A549 an[illegible]C9 cells, the changes in the proliferative, migra[illegible] and invasive abilities were assessed. The in[illegible] action among circ0021205, miRNA-16-5p, a[illegible] the vascular endothelial grow[illegible]A (VEGFA) was verified by Dual-l[illegible]era[illegible]eporter Gene Assay and the Spea[illegible]corre[illegible]on test. At last, the role of cir[illegible]1205/mi[illegible]5p/VEGFA axis in regulating [illegible]og[illegible]CLC was identified.

**RESULTS:** Circ0[illegible]05 was [illegible]gulated in NSCLC tissues a[illegible]ells. Its leve[illegible]ositively correlated to[illegible]ize, tumor i[illegible]metastasis (TNM) s[illegible]ng, [illegible]distant metastasis of NSCLC. Upreg[illegible]lation o[illegible]0021205 predicted worse pro[illegible]sis of NSCL[illegible]ents. The overexpress[illegible]of circ0021205 [illegible]549 and PC9 cells [illegible]lerate[illegible]proliferative, migratory, and inva[illegible]abiliti[illegible]Moreover, circ0021205 could bin[illegible]miR[illegible]6-5p t[illegible]urther mediate VEGFA le[illegible]verexp[illegible]on of miRNA-16-5p or knockdo[illegible]VEGF[illegible]ould reverse the role of overexp[illegible]0021205 in regulating the cel[illegible]behav[illegible]NSCLC.

**C[illegible]CLUSION[illegible]** Circ0021205 aggravates the ma[illegible]ression of NSCLC by binding to m[illegible]o regulate the VEGFA level.

*Words:*
[illegible]021205, MiRNA-16-5p, VEGFA, Non-small cell lung [illegible]ncer (NSCLC).

## [illegible]duction

L[illegible]ncer is the [illegible]g cause of cancer d[illegible]worldwide[1]. About [illegible]5% of lung cancers [illegible]ng to non-small cell lung cancer (NSCLC)[2]. [illegible]spite the rap[illegible]evelopment of imaging ex[illegible]nations, sur[illegible]l procedures, chemotherapy, radioth[illegible]y, the early-stage diagnostic rate [illegible]s increased[3,4]. Moreover, drug targets [illegible]on the mutation and/or amplification of the genes detected in NSCLC (EGFR, [illegible]ALK, ROS1) help to improve the life [illegible]and prognosis of NSCLC patients. Nevertheless, the 5-year survival of NSCLC is still less than 20%[5]. Therefore, an in-depth study of NSCLC has important clinical and public health implications.

In recent years, the study of circular RNA (circRNA) in tumors has become a topic urgent to be investigated[6-8]. CircRNA is a type of RNA that does not have a 5'end cap and a 3'end poly (A) tail. CircRNA forms a loop structure by covalent bonds and is widely present in the eukaryotic cells. Due to its special structure, its potential clinical value in tumor diagnosis, treatment, and prognosis has been highlighted[9-12]. Wan et al[13] reported that cir-ITCH is downregulated in NSCLC tissues and suppresses the malignant proliferation through the Wnt/Catenin pathway. Yao et al[14] showed that circRNA 100876 is markedly upregulated in NSCLC, and closely related to tumor staging. CircRNAs are of significance in the occurrence and progression of NSCLC, and require for fully explorations.

MicroRNA (miRNA) is a single-stranded RNA of approximately 19-21 nucleotides in length. It negatively regulates the target gene expressions by promoting the target mRNA degradation or translation[15]. In recent years, Croce et al[16] have

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found the role of miRNAs in cellular performances and tumor biology. It is noteworthy that miRNA-16-5p is crucial in many types of tumors[17-19]. Liu et al[20] uncovered the role of miRNA-16-5p in NSCLC. It protects lipopolysaccharides (LPS)-induced cell injury in A549 cells, providing a novel target for NSCLC treatment. Therefore, we speculated that miRNA-16-5p could be utilized for the diagnosis, monitor, and treatment of NSCLC. Currently, circRNA is widely explored for its function as a miRNA sponge. As a ceRNA, circRNA could sponge the target miRNA to abolish the inhibitory effect on downstream genes[21].

This study mainly investigated the biological role of circ0021205 in NSCLC and its interaction with miRNA-16-5p.

## Patients and Methods

### Patients and Samples

20 paired tumor tissues and matched adjacent tissues were surgically resected from NSCLC patients treated in the 2nd Affiliated Hospital, Zhejiang University School of Medicine [illegible] May 2016 to October 2018. They did [illegible] ceive preoperative anti-tumor therapy an[illegible] re pathologically diagnosed. The clinical data [illegible] enrolled NSCLC patients were collected. All s[illegible] jects volunteered to participate i[illegible] study an[illegible] signed the written informed [illegible] is study was approved by the Ethics [illegible] mitte[illegible] f the 2nd Affiliated Hospital, Zhej[illegible] Univer[illegible] School of Medicine.

### Cell Culture and [illegible]nsfecti[illegible]

Human bronc[illegible] pithelial ce[illegible] (HBE) and lung can[illegible] es (A549, [illegible], H292, and H358) we[illegible] provid[illegible] Cell Bank (Shanghai, China). The cells we[illegible] tured in Dulbecco's Mo[illegible] d Eagle's Mediu[illegible] MEM; Gibco, Rocky[illegible], MD, [illegible]A) containing 10% fetal bovin[illegible]um (F[illegible] Gibco, Rockville, MD, USA), 100 [illegible] cillin a[illegible]1 mg/mL streptomycin, in a[illegible]5% C[illegible]ncubator.

[illegible] ce[illegible] se[illegible]d in a 6-well plate were tra[illegible]ted us[illegible] pofectamine 2000 (Invitrogen, [illegible]rlsbad, C[illegible], USA). Transfected cells for 24[illegible] vested for *in vitro* experiments.

### [illegible]ll Counting Kit-8 (CCK-8)

[illegible] cells were seeded in the 96-well plate an[illegible]tured overnight. Absorbance (A) at 450 nm was recorded at the appointed time points using the CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan) for depicting the [illegible] curves.

### Quantitative Real Time-Po[illegible]se Chain Reaction (qRT-PCR)

The extraction of tot[illegible]ellular [illegible] performed using TRI[illegible]eagent (Invit[illegible] Carlsbad, CA, USA)[illegible] RNA[illegible]as quali[illegible] using NanoDrop [illegible] (Th[illegible] Fish[illegible] Scientific, Waltham, M[illegible]. Subs[illegible]ently, RNA was sub[illegible]ed to [illegible]se t[illegible]cription using Rever[illegible]ce qPCR [illegible], Osaka, Japan). Th[illegible]ted complem[illegible]ry deoxyribose nu[illegible]ac[illegible]NA) was applied for PCR using the SYBR G[illegible]ethod (TaKaRa, Otsu, Shig[illegible]n).

### [illegible]nswell

[illegible]luted Matr[illegible] was used to pre-coat the T[illegible]ell char[illegible] overnight at 4°C. The cell den[illegible]ted to $5\times10^5$/mL in serum-free medium. [illegible] µL of medium containing 10% FBS and 200 µL of cell suspension were added in the [illegible]l and apical chamber of the 24-well [illegible]ate, [illegible]pectively. 24 h later, the cells were fixed in methanol for 30 min and stained with 0.1% crystal violet for another 30 min. The penetrating cells were observed and photographed using an inverted microscope.

### Western Blot

The total protein was extracted from the cells using radioimmunoprecipitation assay (RIPA) and quantified by bicinchoninic acid (BCA) method (Beyotime, Shanghai, China). The protein sample was loaded for electrophoresis and transferred on polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were blocked in 5% skim milk for 2 hours and subjected to incubation with primary and secondary antibodies. The bands were exposed by enhanced chemiluminescence (ECL) and analyzed by the Image Software (NIH, Bethesda, MD, USA).

### Dual-Luciferase Reporter Gene Assay

The cells were co-transfected with miR-NC/miRNA-16-5p mimics and wild-type/mutant-type vectors using Lipofectamine 2000 for 48 h. The

transfected cells were then lysed for determining the relative luciferase activity (Promega, Madison, WI, USA).

### Statistical Analysis

GraphPad Prism 7.0 (La Jolla, CA, USA) was used for data analyses. The data were expressed as mean ± standard deviation. The intergroup differences were analyzed by the *t*-test. The Chi-square test was performed for assessing the correlation between the circ0021205 level and pathological indexes of the NSCLC patients. Survival analysis was carried out using the Kaplan-Meier method. The relationship between the two genes was evaluated by the Spearman correlation test. $p<0.05$ was considered as statistically significant.

## Results

### Circ0021205 Was Upregulated in NSCLC

Circ0021205 level in NSCLC tissues and adjacent normal tissues was determined. QRT-PCR data showed that circ0021205 level remained higher in NSCLC tissues relative to normal ones (Figure 1A). In patients with NSCLC, patients in stage III-IV expressed higher abundance of circ0021205 relative to those in stage I-II (Figure 1B). Compared with the cells of bronchial epithelial cells, circ0021205 was upregulated in



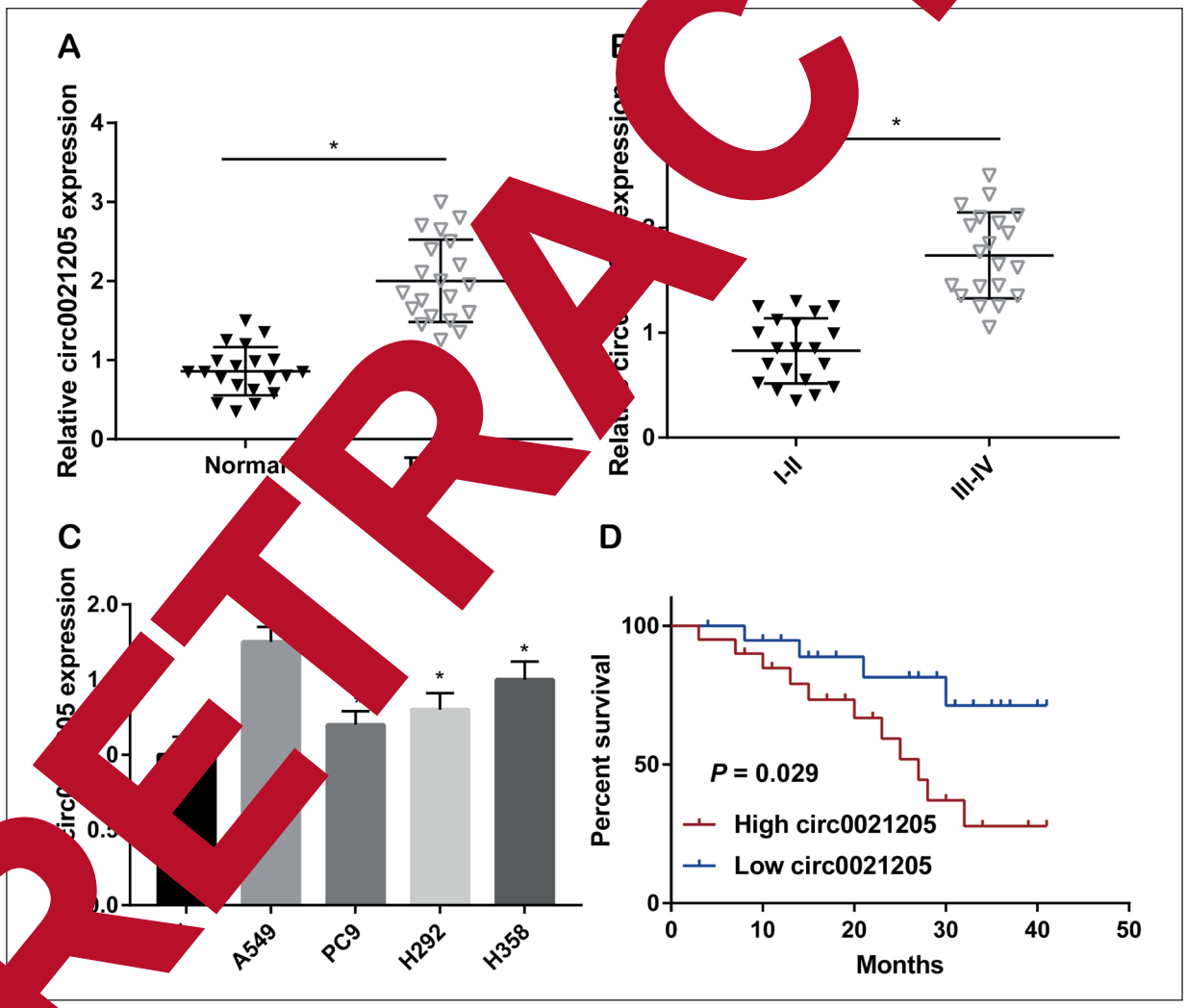


**Figure 1.** Circ0021205 was upregulated in NSCLC. **A,** Relative level of circ0021205 in NSCLC tissues and adjacent normal tissues. **B,** Relative level of circ0021205 in NSCLC patients with stage III-IV and stage I-II. **C,** Relative level of circ0021205 in HBE, A549, PC9, H292, and H358 cells. **D,** Survival analysis of NSCLC patients with high level and low level of circ0021205.

lung cancer cells as well (Figure 1C). A549 and PC9 cells were chosen owing to the highest and lowest level of circ0021205 in the four selected cell lines. The Kaplan-Meier curves indicated worse prognosis in NSCLC patients with a high level of circ0021205 (Figure 1D). By analyzing the clinical data of enrolled patients, it is indicated that circ0021205 level was correlated to tumor size, TNM staging, and lymphatic metastasis of NSCLC patients (Table I). The above results suggested the potential involvement of circ0021205 in the progression of NSCLC.

### *Circ0021205 Accelerated NSCLC to Proliferate, Migrate, and Invade*

To uncover the biological role of circ0021205 in NSCLC, the knockdown and overexpression models of circ0021205 were established in A549 and PC9 cells, respectively. The transfection of si-circ0021205 markedly downregulated circ0021205 level in A549 cells, while the transfection of over-circ0021205 upregulated circ0021205 in PC9 cells (Figure 2A). As the viability curves revealed, the transfection of si-circ0021205 reduced the viability in A549 cells, and conversely, the transfection of over-circ0021205 elevated the viability in the PC9 cells (Figure 2B). The attenuated migratory and invasive abilities were observed in A549 cells transfected with si-circ0021205 and PC9 cells transfected with over-circ0021205, the migratory and invasive capacities were remarkably stimulated (Figure 2C).

### *The Interaction Among Circ0021205, MiRNA-16-5p and Vascular Endothelial Growth Factor A (VEGFA)*

Many studies have shown the function of circRNAs as miRNA sponges thus exerting post-transcriptional regulation on target gene expressions[22,23]. Through TargetScan prediction, the potential binding sites were identified between circ0021205 and miRNA-16-5p, as well as between miRNA-16-5p and VEGFA (Figure 3A). Luciferase activity was reduced after the co-transfection of miRNA-16-5p mimic and circ0021205-WT suggested the binding between circ0021205 and miRNA-16-5p. Specifically, the binding relationship between miRNA-16-5p and VEGFA was identified (Figure 3B). In the collected NSCLC tissues, circ0021205 level was negatively correlated to that of miRNA-16-5p. Additionally, the miRNA-16-5p level was negatively correlated to VEGFA level as well (Figure 3C). The protein level of VEGFA was also negatively regulated by miRNA-16-5p in A549 cells (Figure 3D).

### *Circ0021205 Exerted a Carcinogenic Role in NSCLC Through Targeting MiRNA-16-5p/VEGFA Axis*

A series of rescue experiments were conducted to uncover the role of circ0021205/miRNA-16-5p/VEGFA axis in the progression of NSCLC. The overexpression of circ0021205 downregulated miRNA-16-5p level in A549 cells, which was upregulated by the transfection of miRNA-16-5p mimic (Figure 4A). The increased viability,

**Table I.** The correlation between circ0021205 level and pathological indexes of NSCLC patients (n=20).

| Clinicopathologic features | Number of cases | circ0021205 expression | | *p*-value |
|---|---|---|---|---|
| | | Low (n = 10) | High (n = 10) | |
| Age (years) | | | | 0.074 |
| | 10 | 7 | 3 | |
| | 10 | 3 | 7 | |
| Gender | | | | 0.361 |
| Male | 12 | 5 | 7 | |
| Female | 8 | 5 | 3 | |
| Tumor size | | | | 0.025* |
| TNM | 9 | 7 | 2 | |
| | 11 | 3 | 8 | |
| | | | | 0.007* |
| I-II | 10 | 8 | 2 | |
| III-IV | 10 | 2 | 8 | |
| Lymph metastasis | | | | 0.025* |
| | 11 | 3 | 8 | |
| No | 9 | 7 | 2 | |

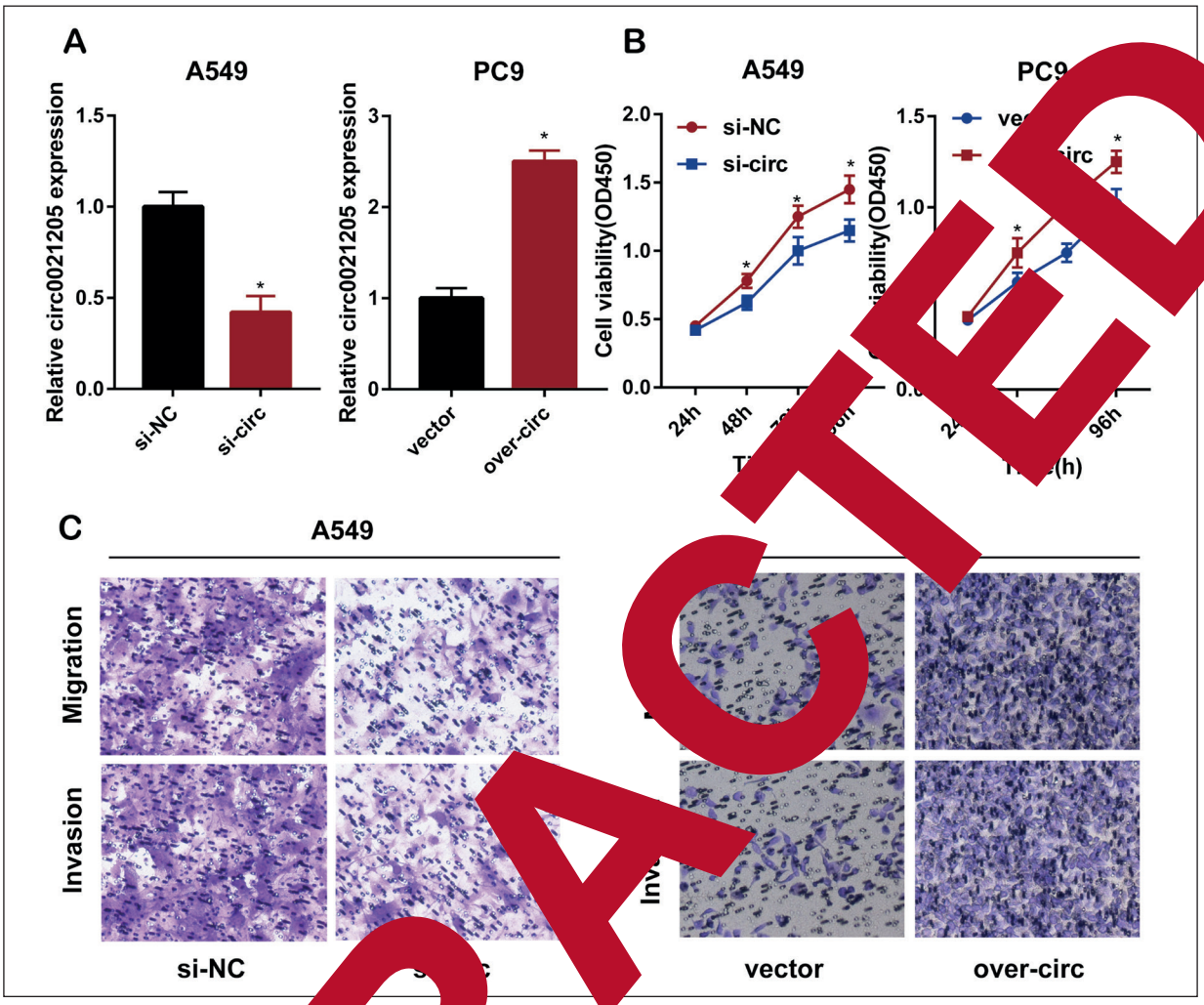


**Figure 2.** Circ0021205 acce NSCL igrate, and invade. **A,** Transfection efficacies of si-circ0021205 and over-circ0021205 in A and PC9 cells transfected with si-NC or si-circ0021205, respectively. **B,** Viability in A549 cells transfected with si-NC or si-circ0021205, and PC9 cells transfected with vecto -circ0021205. **C,** Migration and invasion in A549 cells transfected with si-NC or si-circ0021205, and PC9 cells transfecte ector or over-circ0021205 (magnification: 40×).

migratory an nvasiv numbers in A549 cells over essing circ 5 were partially reversed the co-transfectio miRNA-16-5p mimi si-VEG A (Figures 4B-4D). It is suggest at circ 1205 aggravated the malignant prog SCLC ponging miRNA-16-5p to re VEGF el.

## Discussion

In recent years, the morbidity and mortality of lung cancer have been in the first place throughout the world. NSCLC is the major subtype of lung cancer, accounting for 85%. Owing to the atypical symptoms, the detective rate of early-stage NSCLC is low. Most NSCLC patients are already progressed into an advanced stage at the initial diagnosis. Meanwhile, the poor prognosis of NSCLC is also difficult to be solved[1]. It is of clinical value to find effective early diagnosis and prognostic markers of NSCLC.

CircRNA is a newly discovered non-coding RNA that has been involved in transcriptional and post-transcriptional regulations, thereby mediating the expressions of the eukaryotic genes[24]. A number of studies have shown that circRNA is closely related to the development of various cancers, such as bladder cancer[25], liver cancer[26], colorectal cancer[27], gastric cancer[28], pancreatic cancer[29], and etc. It is necessary to identify

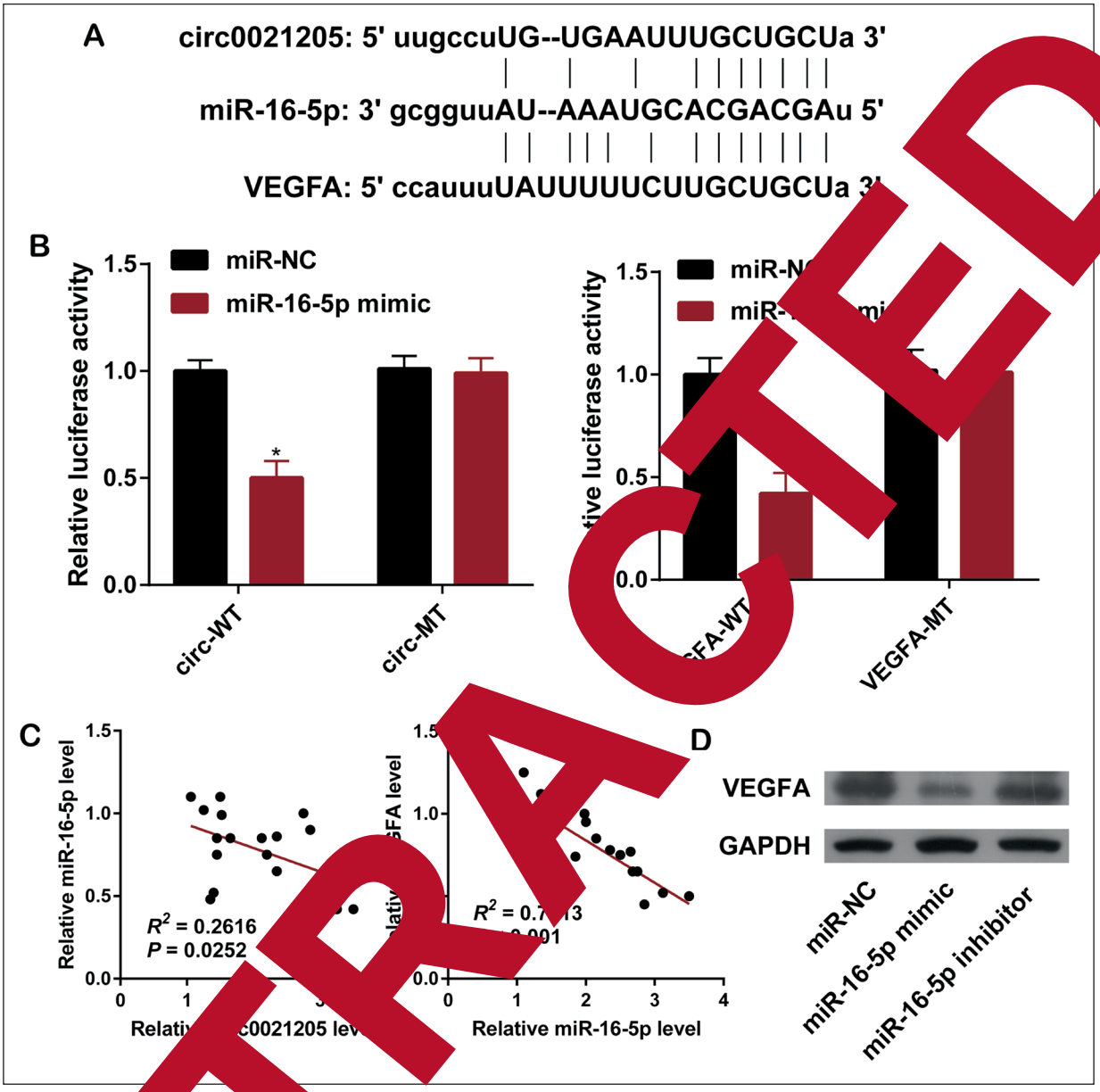


**Figure 3.** T[illegible]nteraction amo[illegible]0021205, miR-16-5p, and VEGFA. **A,** Binding sites of circ0021205/miR-16-5p/VEGFA. **B,** Lucifer[illegible]tivity in A549 c[illegible]ansfected with miR-NC/miR-16-5p mimic and circ0021205-WT/circ0021205-MT (left). Lu[illegible]se activity in A549 cel[illegible]o-transfected with miR-NC/miR-16-5p mimic and VEGFA-WT/VEGFA-MT (right). **C,** A [illegible]ve corr[illegible]n between miR-16-5p and circ0021205 in 20 cases of NSCLC tissues (left). A negative correlation bet[illegible]iR-16-[illegible]d VEGFA in 20 cases of NSCLC tissues (right). **D,** Protein level of VEGFA in A549 cells transfected with [illegible]6-5p mi[illegible]or miR-16-5p inhibitor.



mor[illegible]SCLC-re[illegible]ed circRNAs. In this paper, we [illegible]at circ0021205 was upregulated i[illegible]SCLC t[illegible]ues and cells. High expression of [illegible]0021205 was associated with poor prognosis [illegible]CLC patients. In addition, the overexpression of circ0021205 accelerated NSCLC cells to proliferate, migrate, and invade. It is indicated that circ0021205 can be used as an oncogene in the development of NSCLC.

Some investigations suggested that circRNA regulates tumor progression by sponging miRNAs[30]. CircRNA competitively inhibits the interaction between sponged miRNA and their targets. For example, circ_0003645 exerts a carcinogenic

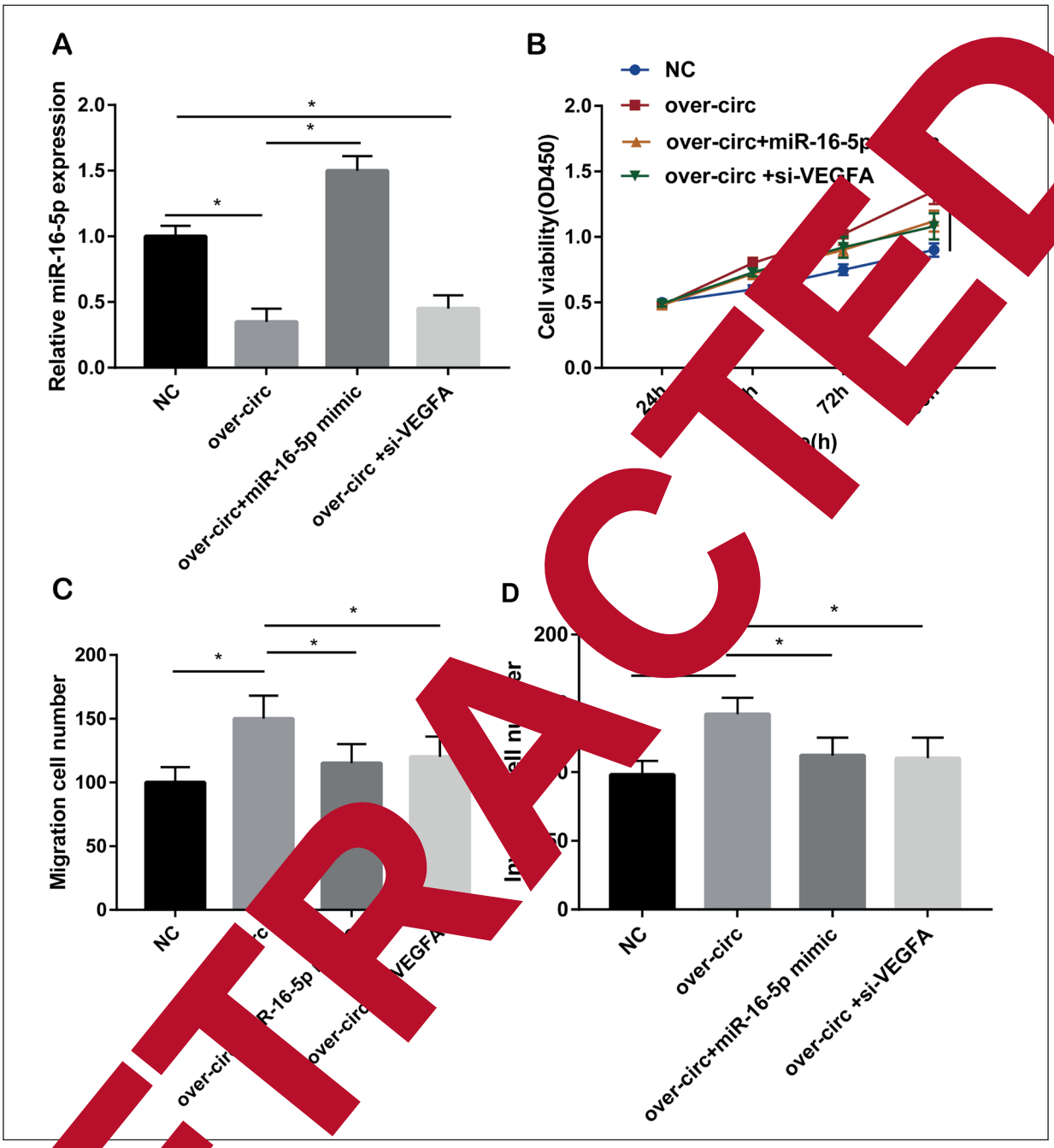




**Fig. 4.** Circ0021205 exerted a carcinogenic role in NSCLC by targeting miR-16-5p/VEGFA axis. A549 cells were transfected with over-circ0021205, over-circ0021205 + miR-16-5p mimic, or over-circ0021205 + si-VEGFA. **A,** Relative level of miR-16-5p. **B,** Viability in A549 cells at 24, 48, 72, and 96 h. **C,** Migratory cell number. **D,** Invasive cell number.

role in NSCLC through the miR-1179/TMEM14A axis. We found that miRNA-16-5p is down-regulated in NSCLC tissues and cells. The over-expression of miRNA-16-5p markedly suppresses proliferation and clonality. This study mainly investigated the role of circ0021205 in NSCLC. We verified the presence of a complementary binding site among circ0021205/miRNA-16-5p/VEGFA by Dual-Luciferase reporter gene assay. Moreover, miRNA-16-5p negatively regulated the expression of VEGFA, while miRNA-16-5p was negatively regulated by circ0021205. Vascular endothelial growth factor (VEGF) has been identified as a key angiogenetic protein that promotes

tumor growth and metastasis[33]. VEGF is a member of the platelet-derived growth factor family, including VEGFA, VEGFB, VEGFC, VEGFD, and VEGFE[34]. Yang et al[35] have found that in NSCLC, miR-140-5p regulates the migratory and invasive abilities of tumor cells by targeting VEGFA. Thus, VEGFA can be considered as a potential target for NSCLC treatment. Our experiments illustrated that the upregulation of miRNA-16-5p or downregulation of VEGFA can reverse the effect of overexpressed circ0021205 on the cellular behaviors of NSCLC cells. Thus, circ0021205 may promote the progression of NSCLC by targeting miRNA-16-5p to regulate the expression of VEGFA.

## Conclusions

Briefly, circ0021205 is upregulated in NSCLC, and closely related to poor prognosis of NSCLC patients. It aggravates the malignant progression of NSCLC by binding to miRNA-16-5p to regulate VEGFA level.

### Conflict of Interest

The Authors declare that they have no conflict of int

## Reference


1) Siegel RL, Miller KD, J. Canc tatistics, 2018. CA Cancer J Cl 68: 7
2) Zheng HE, Wang G ong M, Du W. MicroRNA-495 i s the sion of non-small-cell lung er by targe F4 and inactivating W catenin path ur Rev Med Pharm 8; 22: 7750-7
3) de Jong P ammers reening for lung cancer usi CT: time for i ntation?]. Ned Tijdsch neeskd 2013; 15 9.
4) K hi T, Ta ka N, Matsumoto T, Ueda K, Hoshii nihiro naka T, Hayashi M, Matsunaga N. T find of sma cell lung cancer measur- less l d in the peripheral lung. Jpn ol 2015 67-75.

ng S Minna JD. Comparison be- en con and sequential chemoradiation non-small l lung cancer in vitro. Oncol Lett 307-310.

zman J. Detecting circular RNAs: bioinformatic and experimental challenges. Nat Rev Genet 2016; 17: 679-692.

heski FR, Christoff AP, Margis R. Circular RNAs miRNA sponges and can be used as a new class of biomarker. J Biotechnol 2016; 238: 42-51.

8) Shao Y, Li J, Lu R, Li T, Yang Y, Xiao B, Guo J. Global circular RNA expression profile of human gastric cancer and its clinical significance. C 2017; 6: 1173-1180.
9) Yang F, Liu DY, Guo JT, Ge N, Z Liu X, Wang S, Wang GX, Sun SY. Circular R rc-LDLRAD3 as a biomarker in diagnosis of tic cancer. World J Gastroenterol 201 23: 8 4.
10) Xu T, Wu J, Han P, Zhao ong X. Ci N expression profiles a eatures in hu sues: a study using seq da BMC Ge ics 2017; 18 (Supp 680.
11) Jin X, Guan Y, Shen sstalk i npeting endogen RN k revea e complex molec echani derly ung cancer. Onc t 2017; 8: 91
12) Huang N, Zou Y, Z D, Xiao T. Recent ress ular RNAs in human cancers. Cance Lett 201 8-18.
13) Zhang L, Fan ng ZX, Sun QC, Wang cular RNA-ITCH uppresses lung cancer proliferation via inhibiting the Wnt/β-catenin Pathway. Bio d Res Int 2016; 2016: 1579490.
Yao JT, Zhao iu QP, Lv MQ, Zhou DX, Liao ZJ, Jan KJ. Ove pression of CircRNA_100876 in small c ng cancer and its prognostic val- Pract 2017; 213: 453-456.
15) Fabian MR, Sonenberg N. The mechanics of miRNA-mediated gene silencing: a look under the d of miRISC. Nat Struct Mol Biol 2012; 19: 93.
16) Croce CM, Calin GA. MiRNAs, cancer, and stem cell division. Cell 2005; 122: 6-7.
17) Zhang J, Song Y, Zhang C, Zhi X, Fu H, Ma Y, Chen Y, Pan F, Wang K, Ni J, Jin W, He X, Su H, Cui D. Circulating miR-16-5p and miR-19b-3p as two novel potential biomarkers to indicate progression of gastric cancer. Theranostics 2015; 5: 733-745.
18) Krell A, Wolter M, Stojcheva N, Hertler C, Liesenberg F, Zapatka M, Weller M, Malzkorn B, Reifenberger G. MiR-16-5p is frequently down-regulated in astrocytic gliomas and modulates glioma cell proliferation, apoptosis and response to cytotoxic therapy. Neuropathol Appl Neurobiol 2018. doi: 10.1111/nan.12532. [Epub ahead of print].
19) Chen SS, Tang CH, Chie MJ, Tsai CH, Fong YC, Lu YC, Chen WC, Lai CT, Wei CY, Tai HC, Chou WY, Wang SW. Resistin facilitates VEGF-A-dependent angiogenesis by inhibiting miR-16-5p in human chondrosarcoma cells. Cell Death Dis 2019; 10: 31.
20) Liu GP, Wang WW, Lu WY, Shang AQ. The mechanism of miR-16-5p protection on LPS-induced A549 cell injury by targeting CXCR3. Artif Cells Nanomed Biotechnol 2019; 47: 1200-1206.
21) Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, Maier L, Mackowiak SD, Gregersen LH, Munschauer M, Loewer A, Ziebold U, Landthaler M, Kocks C, le Noble F, Rajewsky N. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 2013; 495: 333-338.

22) Yang M, Li G, Fan L, Zhang G, Xu J, Zhang J. Circular RNA circ_0034642 elevates BATF3 expression and promotes cell proliferation and invasion through miR-1205 in glioma. Biochem Biophys Res Commun 2019; 508: 980-985.

23) Li Y, Wan B, Liu L, Zhou L, Zeng Q. Circular RNA circMTO1 suppresses bladder cancer metastasis by sponging miR-221 and inhibiting epithelial-to-mesenchymal transition. Biochem Biophys Res Commun 2019; 508: 991-996.

24) Lasda E, Parker R. Circular RNAs co-precipitate with extracellular vesicles: a possible mechanism for circRNA clearance. PLoS One 2016; 11: e0148407.

25) Yang C, Yuan W, Yang X, Li P, Wang J, Han J, Tao J, Li P, Yang H, Lv Q, Zhang W. Circular RNA circ-ITCH inhibits bladder cancer progression by sponging miR-17/miR-224 and regulating p21, PTEN expression. Mol Cancer 2018; 17: 19.

26) Yu J, Xu QG, Wang ZG, Yang Y, Zhang L, Ma JZ, Sun SH, Yang F, Zhou WP. Circular RNA cSMARCA5 inhibits growth and metastasis in hepatocellular carcinoma. J Hepatol 2018; 68: 1214-1227.

27) Zeng K, Chen X, Xu M, Liu X, Hu X, Xu T, Sun H, Pan Y, He B, Wang S. CircHIPK3 promotes colorectal cancer growth and metastasis by sponging miR-7. Cell Death Dis 2018; 9: 417.

28) Han D, Li J, Wang H, Su X, Hou J, Gu Y, C[illegible] C, Lin Y, Liu X, Huang M, Li N, Zhou W, Yu [illegible]. Circular RNA circMTO1 acts as the sp[illegible] microRNA-9 to suppress hepatocellular [illegible]noma progression. Hepatology 2017; 66: 1151-1164.

29) Huang WJ, Wang Y, Liu S, Yang J, Guo SX, Wang L, Wang H, Fan YF. Silencing circular RNA hsa_circ_0000977 suppresses pancreatic [illegible]enocarcinoma progression by sti[illegible]g 874-3p and inhibiting PLK1 exp[illegible]on. Cance Lett 2018; 422: 70-80.

30) Hansen TB, Jensen TI, Clausen B[illegible]sen JB, Finsen B, Damgaard CK, Kjems J. Natur[illegible] circles function as efficient mi[illegible]A spon[illegible]tur 2013; 495: 384-388.

31) An J, Shi H, Zhang M[illegible]ng S. E[illegible]tion of ci[illegible] RNA circ_0003645 [illegible]casts u[illegible]rable prognosis and facilitates ce[illegible]assi[illegible]a miR-1[illegible]TME-M14A pathwa[illegible]no[illegible]l lung [illegible]r. Biochem Biophy[illegible]s Com[illegible]19; 51[illegible]-925.

32) Ke Y, Zha[illegible] Xiong J, Cao [illegible]egulation of miR-16[illegible] growth and m[illegible]y by targeting HDG[illegible]no[illegible] cell lung cancer cells. FEBS Lett 2[illegible]3; 587: [illegible]157.

33) Fu P, Cheng W, [illegible]Xu X, Yi X, Tang C, Wang [illegible], Zhang Z. Imp[illegible] vascular endothelial growth factor gene-gene and gene-smoking interaction and haplotype combination on bladder cancer risk [illegible]hinese population. Oncotarget 2017; 8: 2292[illegible]935.

3[illegible]nikaruk S, [illegible] M, Laïn G, Déléris G. Vascular [illegible] growth factor (VEGF), an emerging [illegible]r cancer chemotherapy. Curr Med Chem Anticancer Agents 2003; 3: 95-117.

[illegible]ang P, Xiong J, Zuo L, Liu K, Zhang H. MiR1405p [illegible]tes cell migration and invasion of nonsmall [illegible]lung cancer cells through targeting VEGFA. Mol Med Rep 2018; 18: 2866-2872.