A Regulatory Axis of circ_0008193/miR-1180-3p/TRIM62 Suppresses Proliferation, Migration, Invasion, and Warburg Effect in Lung Adenocarcinoma Cells Under Hypoxia

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Background: Expression profiles of circular ribonucleic acids (circRNAs) have been recently reported in lung cancers including lung adenocarcinoma (LUAD). Hypoxia is a hallmark of lung cancers. However, the role of hsa_circ_0008193 (circ_0008193) in LUAD under hypoxia remains to be illuminated.

Material/Methods: Gene expression levels were detected using real-time quantitative polymerase chain reaction and western blotting. Cell proliferation, migration, invasion, and Warburg effect were detected using 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide assay, transwell assays, special kits, and xenograft experiments. The relationship among circ_0008193, micro (mi)RNA (miR)-1180-3p, and tripartite motif containing 62 (TRIM62) was confirmed by dual-luciferase reporter assay and RNA immunoprecipitation.

Results: Expression of circ_0008193 was downregulated in human LUAD tumor tissues and cell lines (A549 and H1975), accompanied by miR-1180-3p upregulation and TRIM62 downregulation. Moreover, circ_0008193 downregulation was correlated with tumor size and lymph node metastasis. Functionally, circ_0008193 overexpression inhibited cell viability, glucose uptake, lactate production, migration, and invasion, as well as expression of hexokinase II, lactate dehydrogenase A, matrix metalloproteinase 2 (MMP2), and MMP9 in hypoxic LUAD cells in vitro. Furthermore, tumor growth of A549 cells in vivo was also hindered by circ_0008193 overexpression. Mechanically, circ_0008193 regulated TRIM62 expression via sponging miR-1180-3p, and TRIM62 was targeted by miR-1180-3p. Both miR-1180-3p upregulation and TRIM62 downregulation could abolish the suppressive role of circ_0008193 in LUAD cells.

Conclusions: Upregulating circ_0008193 inhibited LUAD cell proliferation, migration, invasion, and Warburg effect under hypoxia in vitro and in vivo through regulation of the miR-1180-3p/TRIM62 axis.

MeSH Keywords: Adenocarcinoma • Lung Neoplasms • Pneumonia

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Background

Lung adenocarcinoma (LUAD) is the common subtype of non-small-cell lung cancer (NSCLC), and LUAD occurs mostly in the peripheral airways of the lung [1]. According to global cancer statistics, LUAD accounts for almost 40% of lung cancer deaths [2]. Because the diagnosis of lung cancer occurs mostly at advanced stages [3], prognosis of patients with lung cancers has not dramatically improved in recent years, even though treatment has made good advances [4]. Thus, discovering new biomarkers for the identification of early LUAD is imperative. Circular ribonucleic acids (circRNAs) are subcategorized as a subtype of endogenous noncoding RNA with a circular form. Essentially, circRNAs are the back-splicing products of precursor messenger RNAs (mRNAs) [5]. Notably, the closed structure of circRNAs makes them more stable to exonuclease digestion [6], thus universally existing in tissues and circulating body fluids such as serum and urine. Previous evidence has revealed the involvement of circRNAs in human diseases [7]. Multiple expression profiles of circRNAs have also been uncovered in tissues [8], peripheral whole blood [9], and plasma exosomes [10] from LUAD patients. Several circRNAs are suggested to be noninvasive diagnostic biomarkers for LUAD [11,12]. Here, we aimed to investigate the expression and role of hsa_circ_0008193 (circ_0008193) in LUAD cells, as well as its competing endogenous RNA (ceRNA) mechanism [13]. Physically, circRNAs are mostly regarded as micro (mi)RNA sponges. Smoking trends have probably dictated global epidemiology of lung cancers [14], and miRNA (miR)-1180-3p is one of the top smoking-associated miRNAs that is associated with pulmonary function [15]. Tripartite motif containing 62 (TRIM62; also named as DEAR1) is a tumor suppressor in cancers, including lung cancer [16–18]. Hypoxia has been well recognized to be one tumor-specific microenvironment, including in lung cancers [19]. Collectively, we attempted to measure the interaction among circ_0008193, miR-1180-3p, and TRIM62 in LUAD cells under hypoxia.

Material and Methods

Patients' information and tissue sample acquisition

A total of 53 paired surgical pathology specimens including tumor tissues and paired peripheral normal lung tissues was obtained from patients with LUAD in Hainan General Hospital. The specimens were snap-frozen and stored at −80°C until use. The patients had primary LUAD and received no neoadjuvant therapy before this surgery. The clinicopathological features were collected and presented in Table 1, and survival data were traced after surgery. This study was approved by the Ethics Committee of Hainan General Hospital in accordance with the Declaration of Helsinki, and written informed consent was received from every patient before sample collection.

Table 1. Correlation between hsa_circ_0008193 (FAM120A) level and pathological indexes of lung adenocarcinoma patients.

| Parameter                  | Case       | hsa_circ_0008193 expression | P value* |
|----------------------------|------------|-----------------------------|----------|
|                            |            | Low (n=27)                  | High (n=26) |          |
| Age (years)                |            |                             |          |
| ≤60                        | 32         | 18                          | 14        | 0.5009   |
| >60                        | 21         | 9                           | 12        | 0.4999   |
| Gender                     |            |                             |          |
| Male                       | 23         | 10                          | 13        | 0.9198   |
| Female                     | 30         | 17                          | 13        |          |
| Smoking                    |            |                             |          |
| Yes                        | 29         | 14                          | 15        | 0.5076   |
| No                         | 24         | 10                          | 14        |          |
| Tumor size                 |            |                             |          |
| ≤3 cm                      | 20         | 16                          | 4         | 0.0026*  |
| >3 cm                      | 33         | 11                          | 22        |          |
| Lymph node metastasis      |            |                             |          |
| No                         | 35         | 24                          | 11        | 0.0010*  |
| Yes                        | 18         | 3                           | 15        |          |

* P<0.05. * Chi-square test.
The patients were followed up for 5 years by telephone and the overall survival time was defined as the time from the surgery to the last follow-up or death.

**Cells and cell culture**

Human LUAD cell lines A549 (cat: C0016002) and H1975 (cat: C0016013) and normal human lung epithelial cell line BEAS-2B (cat: T000701) were purchased from AddexBio (San Diego, CA, USA). These cells were cultured in Roswell Park Memorial Institute media 1640 (RPMI; HyClone, Logan, UT, USA) with 10% fetal bovine serum (FBS; HyClone) at 37°C and 5% CO₂.

**Cell transfection**

For overexpression of circ_0008193, the full-length complementary deoxyribonucleic acid (cDNA) of circ_0008193 was cloned into pLCDH-ciR vector (GenePharma, Shanghai, China). The mir-1180-3p mimic (5'-UGUGUGGUGGCUGGCUCUU-3'), small interfering (si)RNA against TRIM62 (si-TRIM62; sense 5'-CCCUAGAAGGUGUCCAUGA-3' and antisense 5'-CGCACAAGGUUCCAGU-3'), and their negative controls miR-NC mimic (5'-GUCCAGUGAAUUCCAG-3') and si-NC (sense 5'-UUCUCCGAACGUGUGACGU-3' and antisense 5'-ACGUGACAGGUGUCCAATG-3') were provided by GenePharma as well. A549 and H1975 cells were exogenously administered with these nucleotides using Lipofectamine™3000 reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s instructions.

**Hypoxia treatment**

A549 and H1975 cells were passaged and grown to 70% confluence. Cells were then transferred in a humidified hypoxic chamber (Coy Laboratory Products, Inc, Grass Lake, MI, USA) for incubation with 1% O₂, 5% CO₂, and 94% N₂ for 48 h. The control cells were cultivated in normoxic condition (20% O₂, 5% CO₂, and 75% N₂).

**Real-time quantitative polymerase chain reaction (RT-qPCR)**

Total RNA in tissues and cells was extracted using RNAiso Plus (Takara, Shiga, Japan). This RNA sample (2 µg) was treated with RNase R (2 U/µg; Epicentre Technologies, Madison, WI, USA) for 30 min at 37°C or not. The cDNA was generated using cDNA reagent (Invitrogen, Carlsbad, CA, USA) and the results were presented according to threshold cycle method, normalized to internal controls glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or U6. The paired primer of circ_0008193 was 5'-ACCTCCTTGATACCATAGC-3' (forward) and 5'-ATTCACTGGCCGATGTA-3' (reverse), FAM120A was 5'-AGGGCGAAGTCCAACCTAT-3' (forward) and 5'-CTGGTCCTCCGACAGAC-3' (reverse), miR-1180-3p was 5'-CAGAACACGCAATACACAG-3' (forward) and 5'-GCCTCAGGATGCTAAT-3' (reverse), TRIM62 was 5'-TTGATCCAAAGATGTGACATG-3' (forward) and 5'-GTGACCCGTTGGACTGG-3' (reverse), GAPDH was 5'-CAGCCAGAAATACAAACAG-3' (forward) and 5'-GACTGACTTCGAAACGGCC-3' (reverse), and U6 was 5'-CTGCCTCAGGACACATATCT-3' (forward) and 5'-ACGCTTCAGAATTTGGCTGTC-3' (reverse).

**Analysis of glucose consumption and lactate production**

Hypoxia-treated A549 and H1975 cells were starved in 0.1% glucose-free RPMI containing 0.1% FBS for 16 h. Then, these cells was added 2-(N-[7-nitrobenz-2-oxa-1,3-diazol-4-yl] amino)-2-deoxy-d-glucose (5 μM) for 30 min, and the fluorescence intensity was read using a microplate reader (Clario Star; BMG Labtech, Ortenberg, Germany). The concentration of lactate was measured using lactate assay kit II (Invitrogen) before collection of the cell extract of hypoxic A549 and H1975 cells.

**Western blotting**

For total protein determination, tissues and cells were lysed in an appropriate volume of radioimmunoprecipitation (RIPA) buffer (Roche Diagnostics, Mannheim, Germany) on ice, and the supernatant was collected after centrifugation at 15 000×g for 20 min. Twenty micrograms of protein samples were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA). After incubation of the special primary antibodies for 8 h at 4°C including hexokinase II (HK2; ab227198, 1: 10 000), lactate dehydrogenase A (LDHA; ab125683, 1: 2 000), matrix metalloproteinase 2 (MMP2;
Expression of circ_0008193 was downregulated in human LUAD tumor tissues and cell lines

Expression of circ_0008193 in LUAD was measured, and RT-qPCR data showed a lower expression of circ_0008193 in both LUAD tumor samples (n=53) and cell samples (A549 and H1975) (Figure 1A, 1B). Moreover, circ_0008193 expression was not affected by RNase R treatment, whereas FAM120A, the parent gene of circ_0008193, was dramatically decreased by RNase R digestion (Figure 1C, 1D). Clinically, this downregulation of circ_0008193 was associated with large tumor size and lymph node metastasis as well as overall survival of LUAD patients (Table 1, Figure 1E). Hypoxia-inducible factor 1 (HIF1) was the main oxygen sensor that regulated the adaptation to intratumoral hypoxia [20], and its subunit HIF1 alpha (HIF1A) was a pivotal pathway in hypoxic tumor growth and progression [21]. Thus, RT-qPCR also indicated that hypoxia-induced HIF1A expression at high levels in A549 and H1975 cells (Supplementary Figure 1A), accompanied by an even lower expression of circ_0008193 than in normoxic cells (Figure 1F, 1G).
These data showed a downregulation of circ_0008193 in LUAD tissues and cells, suggesting a potential role of circ_0008193 in hypoxic LUAD cells.

**Upregulation of circ_0008193 suppressed cell proliferation, migration, invasion, and Warburg effect in LUAD cells under hypoxia in vitro**

Next, the effect of circ_0008193 in LUAD cells under hypoxia was investigated in vitro. In response to hypoxia treatment, circ_0008193 was silenced in A549 and H1975 cells (Figure 2A), and cell viability was highly induced according to MTT assay (Figure 2B). The activation of oxygen-independent glycolysis has been well known in lung cancers and is called the Warburg effect [22]. Commercial kits revealed that glucose uptake and lactate production were facilitated by hypoxia treatment in A549 and H1975 cells (Figure 2C, 2D); western blotting determined that HK2 and LDHA expression was elevated in hypoxia-treated A549 and H1975 cells (Figure 2E, 2F). Hypoxia always stimulated tumor metastasis [23]; therefore, cell migration and invasion were measured. Transwell assay showed that migration and invasion cell numbers were significantly higher in A549 and H1975 cells under hypoxic condition (Figure 2G–2J), along with increased MMP2 and MMP9 expression (Figure 2K, 2L). The aforementioned outcomes together indicated that hypoxia contributed to LUAD cell proliferation, migration, invasion, and Warburg effect in vitro. However, with transfection of circ_0008193 overexpression vectors, RT-qPCR analysis revealed a restoration of circ_0008193 in hypoxic A549 and H1975 cells after transfection with circ_0008193 overexpression vectors (Figure 2A), and this upregulation caused a diminishing effect on cell viability, glucose uptake, lactate production, numbers of migrating and invading cells, and levels of HK2, LDHA, MMP2, and MMP9 (Figure 2B–2L). Moreover, HIF1A upregulation in hypoxia-treated A549 and H1975 cells was suppressed with circ_0008193 overexpression, and this suppression was then counteracted by restoring miR-1180-3p (Supplementary Figure 1B). Thus we propose a tumor-suppressive role of circ_0008193 in hypoxic LUAD cells in vitro.

**Circ_0008193 could regulate miR-1180-3p expression via physically binding**

Previously, studies showed that circRNAs could serve as ceRNAs to sponge miRNAs [24]. Here, we attempted to confirm a potential direct relationship between circ_0008193 and miRNAs, and miR-1180-3p possessed the highest score according to circBank (http://www.circbank.cn/hsa_0008193-mirnas) prediction algorithm. The putative binding sites in circ_0008193 were presented as shown in Figure 3A. Dual-luciferase reporter assay manifested a significant decrease of relative luciferase activity in A549 cells introduced with circ_0008193-WT and miR-1180-3p mimic (Figure 3B, 3C); in contrast, circ_0008193 and miR-1180-3p were concurrently enriched in Ago2-RIP (Figure 3D, 3E). Expression of miR-1180-3p was also suppressed in circ_0008193-overexpressed A549 and H1975 cells (Figure 3F, 3G). The aforementioned data suggest a target relationship between circ_0008193 and miR-1180-3p. Expression of miR-1180-3p in LUAD was measured, and RT-qPCR data showed a higher expression of miR-1180-3p...
Figure 2. Effect of circ_0008193 overexpression in human lung adenocarcinoma (LUAD) cells under hypoxia in vitro. (A) Real-time quantitative polymerase chain reaction (RT-qPCR)-confirmed circ_0008193 level in hypoxic A549 and H1975 cells transfected with vectors carrying circ_0008193 or not (vector or circ_0008193). (B) 3-(4, 5-Dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) assay evaluated cell viability; (C, D) special kits assessed glucose and lactate concentrations; (E, F) western blotting measured hexokinase II (HK2) and lactate dehydrogenase A (LDHA) expression; (G–J) transwell determined numbers of migrating and invading cells; (K, L) western blotting also measured matrix metalloproteinase 2 (MMP2) and MMP9 levels in transfected A549 and H1975 cells after hypoxia treatment for 48 h. *** P<0.001 from three independent experiments.
Figure 3. Relationship between circ_0008193 and micro (mi)RNA (miR)-1180-3p in human lung adenocarcinoma (LUAD) tissues and cells. (A) Wild type of circ_0008193 (circ_0008193-WT) was predicted to contain potential binding sites of miR-1180-3p. (B, C) Dual-luciferase reporter assay identified luciferase activity of vectors carrying circ_0008193-WT or mutant type circ_0008193-MUT. (D, E) Ribonucleic acid (RNA) immunoprecipitation validated the enriched levels of circ_0008193 and miR-1180-3p. (F, G) Real-time quantitative polymerase chain reaction (RT-qPCR) examined miR-1180-3p level in A549 and H1975 cells in the presence of vector or circ_0008193. RT-qPCR measured miR-1180-3p expression status in (H) tissues from patients (n=53) with LUAD and (I) LUAD cell lines. (J, K) RT-qPCR detected miR-1180-3p level in hypoxic A549 and H1975 cells. *** P<0.001 from three independent experiments.
in both LUAD tumor samples (n=53) and cell samples (A549 and H1975) (Figure 3H, 3I), and an even higher level in hypoxia-treated A549 and H1975 (Figure 3I, 3K). These results indicated a promising association between circ_0008193 and miR-1180-3p in tumor cell progression in LUAD.

**Inhibited miR-1180-3p mediated the tumor-suppressive role of circ_0008193 in LUAD cells under hypoxia in vitro**

The impact of miR-1180-3p expression on the role of circ_0008193 in LUAD cells was verified as follows. Hypoxic A549 and H1975 cells were pretransfected with circ_0008193 alone or combined with miR-1180-3p mimic. Endogenous miR-1180-3p expression was highly induced by hypoxia treatment, which was depressed by circ_0008193 ectopic expression (Figure 4A); this inhibition of circ_0008193 on miR-1180-3p expression was rescued with exogenous administration of miR-1180-3p mimic. Downregulation of miR-1180-3p mediat- ed by circ_0008193 transfection resulted in low abilities of cell migration and invasion (Figure 4B–4E). Moreover, introduction of miR-1180-3p mimic inhibited hypoxia-treated A549 and H1975 cells (Figure 4B–4F). These results indicated that elevated circ_0008193 expression could reverse the decrease in cell migration and invasion in hypoxic A549 and H1975 cells, which was mediated by downregulated miR-1180-3p expression during hypoxic conditions. Furthermore, inhibition of miR-1180-3p expression could be suppressed by overexpression of circ_0008193 (Figure 4G–4I), suggesting that hypoxic conditions possibly suppressed the tumor-suppressive role of miR-1180-3p on tumor cells migration and invasion.
miR-1180-3p mimic could in turn block the suppressive role of circ_0008193 in cell proliferation, migration, invasion, and Warburg effect of A549 and H1975 cells (Figure 4B–4J). The inhibition of circ_0008193 upregulation on HIF1A expression in LUAD cell lines. (H, I) Western blotting detected TRIM62 level in hypoxic A549 and H1975 cells. (J, K) Western blotting detected TRIM62 level in A549 and H1975 cells in the presence of vector or circ_0008193 and copresence of miR-1180-3p. *** P<0.001 from three independent experiments.

TRIM62 was a downstream target for miR-1180-3p and was modulated by circ_0008193 via miR-1180-3p

We further explored the downstream functional gene of miR-1180-3p. According to TargetScan (http://www.targetscan.org/mir-1180-3p-TRIM62) prediction algorithm, TRIM62 3’-UTR possessed the potential binding sites of miR-1180-3p (Figure 5A). Dual-luciferase reporter assay manifested a significant decrease in TRIM62 luciferase activity in A549 cells treated with miR-1180-3p mimic compared to miR-NC (Figure 5B–5D).

Figure 5. Relationship between miR-1180-3p and tripartite motif containing 62 (TRIM62) in human lung adenocarcinoma (LUAD) tissues and cells. (A) Wild type of TRIM62 3’-untranslated region (TRIM62 3’-UTR-WT) was predicted to contain potential binding sites of miR-1180-3p. (B, C) Dual-luciferase reporter assay identified luciferase activity of vectors carrying TRIM62 3’-UTR-WT or mutant type (TRIM62 3’-UTR-MUT). (D) Western blotting measured TRIM62 expression in LUAD cell lines. (H, I) Western blotting detected TRIM62 level in hypoxic A549 and H1975 cells. (J, K) Western blotting detected TRIM62 level in A549 and H1975 cells in the presence of vector or circ_0008193 and copresence of miR-1180-3p. *** P<0.001 from three independent experiments.
Expression of TRIM62 in LUAD was also measured, and a direct target relationship between miR-1180-3p and TRIM62 was also suppressed in miR-1180-3p-overexpressed A549 and H1975 cells (Figure 5D, 5E). The aforementioned data suggest that miR-1180-3p together with circ_0008193 could inhibit tumor cell progression in LUAD.

**Figure 6.** Impact of tripartite motif containing 62 (TRIM62) expression on the role of circ_0008193 in human lung adenocarcinoma (LUAD) cells under hypoxia in vitro. (A) Western blotting confirmed TRIM62 level in hypoxic A549 and H1975 cells cotransfected with circ_0008193 and small interfering ribonucleic acid (siRNA) against TRIM62 (si-TRIM62) or its control si-NC. (B) 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay evaluated cell viability; (C, D) special kits assessed glucose and lactate concentrations; (E, F) western blotting measured hexokinase II (HK2) and lactate dehydrogenase A (LDHA) expression; (G, H) transwell determined numbers of migrating and invading cells; (I, J) western blotting also measured metalloproteinase (MMP)2 and MMP9 levels in transfected A549 and H1975 cells after hypoxia treatment for 48 h. * P<0.05 and *** P<0.001 from three independent experiments.

of relative luciferase activity of the vector carrying TRIM62 3’-UTR-WT in A549 and H1975 cells introduced with miR-1180-3p mimic (Figure 5B, 5C). Expression of TRIM62 protein was also suppressed in miR-1180-3p-overexpressed A549 and H1975 cells (Figure 5D, 5E). The aforementioned data suggest a direct target relationship between miR-1180-3p and TRIM62. Expression of TRIM62 in LUAD was also measured, and western blotting data showed a lower expression of TRIM62 in both LUAD tumor samples (n=53) and cell samples (A549 and H1975) (Figure 5F, 5G), and an even lower expression of TRIM62 in hypoxia-treated A549 and H1975 (Figure 5H, 5I). TRIM62 expression was higher in circ_0008193-upregulated cells, and this upregulation was abrogated by the presence of miR-1180-3p mimic (Figure 5J, 5K). These results indicated a promising association among circ_0008193, miR-1180-3p, and TRIM62 in tumor cell progression in LUAD.
Elevated TRIM62 mediated the suppressive role of circ_0008193 in LUAD cells under hypoxia in vitro

The impact of TRIM62 expression on the role of circ_0008193 in LUAD cells was verified as well. Hypoxic A549 and H1975 cells were pretransfected with circ_0008193 alone or combined with si-TRIM62. Endogenous TRIM62 expression was expressed at low levels in response to hypoxia, which was elevated by circ_0008193 ectopic expression; this enhancement of circ_0008193 on TRIM62 expression was counteracted by exogenous administration of si-TRIM62 (Figure 6A). TRIM62 upregulation mediated by circ_0008193 overexpression via transfection suppressed hypoxia-induced cell viability (Figure 6B), glucose consumption (Figure 6C), lactate production (Figure 6D), HK2 and LDHA expression (Figure 6E, 6F), and migrating and invading cells (Figure 6G, 6H), as well as MMP2 and MMP9 expression (Figure 6I, 6J) in A549 and H1975 cells. In addition, restoring TRIM62 expression could in turn block the suppressive role of circ_0008193 in cell proliferation, migration, invasion, and Warburg effect of A549 and H1975 cells (Figure 6B–6J). The inhibition of circ_0008193 upregulation on HIF1A expression was also abrogated by silencing TRIM62 (Supplementary Figure 1C). Therefore, we proposed that the suppressive role of circ_0008193 in LUAD cells under hypoxia in vitro was dependent on TRIM62 upregulation.

Overexpressed circ_0008193 hindered tumor growth of LUAD cells in vivo

In vivo, we intended to study the effect of circ_0008193 on tumorigenicity of A549 cells in mice. As shown in Figure 7A–7C, A549 cells induced neoplasms in nude mice; the tumor volume and weight of circ_0008193 group (n=4) were less than the vector group (n=4). Notably, the neoplasms injected with circ_0008193 overexpression vectors exhibited significantly higher circ_0008193 (Figure 7D) and TRIM62 (Figure 7F, 7G) and lower miR-1180-3p (Figure 7E). These outcomes indicated that circ_0008193 upregulation hindered tumor growth of A549 cells in vivo by downregulating miR-1180-3p and upregulating TRIM62.

Discussion

Hypoxia always occurs in the pathology of cancers. In response to hypoxia, a large number of target genes are activated, and HIF1A is one key pathway under hypoxic signaling and adaptation through targeting special genes [25] such as forkhead box C1 and glycogen branching enzyme [26,27]. Dozens of circRNAs have also been declared to be significantly abundantly altered upon hypoxia in cervical, breast, and lung cancer cells in vitro [28]. Moreover, the circRNA expression profile was comprehensively compared in A549 cells under normoxia and hypoxia [29]. Since hypoxia is crucial to the initiation and progression of tumor metastasis, several circRNAs have been demonstrated to participate in LUAD cell migration and invasion. For example, circ_0000211 and circ_0014130 were shown to facilitate LUAD cell endothelial-to-mesenchymal transition (EMT), wound-healing ability, and transwell migration and invasion properties by positively modulating HIF1A [30,31]. Upregulating circCCDC66 was associated with hypoxia-induced...
EMT and epidermal growth factor receptor resistance in LUAD cells [32]. Here, we explored the effect of circ_0008193 on cell proliferation, migration, invasion, and Warburg effect in LUAD cells via the miR-1180-3p/TRIM62 axis.

We observed that circ_0008193 was significantly downregulated in LUAD tumor tissues and cell lines, as well as in hypoxia-treated A549 and H1975 cells (with HIF1A upregulation). These data agreed with a previous study [29]. In this study, we further examined the biologic role of circ_0008193 in LUAD cells. As a result, we indicate that overexpression of circ_0008193 could inhibit hypoxia-induced cell viability, glucose consumption, lactate production, and HK2 and LDHA expression, as well as diminish transwell migration and invasion abilities and MMP2 and MMP9 expression. By these observations, this study suggests that clinically, circ_0008193 is a novel promising target of hypoxic LUAD. The growth of neoplasms mediated by A549 cells was restrained by circ_0008193 overexpression in vitro. With inspiration from the prediction of target miRNAs by Cheng et al. [29], we chose miR-1180-3p for further confirmation. Here, we observed that HIF1A upregulation in hypoxia-treated A549 and H1975 cells led to circ_0008193 inhibition (as well as miR-1180-3p promotion), and overexpressing circ_0008193 caused HIF1A downregulation (accompanied by miR-1180-3p deficiency). These findings suggest that expression of circ_0008193 and miR-1180-3p in LUAD is a HIF1A-dependent mechanism.

MiR-1180-3p was suggested to be one essential plasma biomarker to distinguish gastric cancer from benign gastritis, and to distinguish early gastric cancer from advanced gastric cancer [33]. This miRNA was also used to discriminate hepatocellular carcinoma (HCC) occurrence after direct antiviral therapy [34]. In lung cancer, miR-1180-3p was previously discovered to be upregulated in the sera and tumor tissues from lung cancer patients and was correlated with higher TNM stage and poor overall survival [35]. Here, our data supported the upregulation of miR-1180-3p in LUAD tissues and cell lines, and even higher expression of miR-1180-3p was observed in hypoxia-treated A549 and H1975 cells (with HIF1A upregulation). Similarly, this miRNA was a common target for different ceRNAs (such as long noncoding RNA DGCR5 [35] and circ_0008193) to affect the proliferation, migration, and invasion of NSCLC cells. Furthermore, we validated that miR-1180-3p upregulation was one molecular mechanism of hypoxia-induced cell proliferation, migration, invasion, and Warburg effect. Notably, TRIM62 was identified as a functional target gene of miR-1180-3p. In addition, miR-1180-3p could exert anti-apoptotic function in HCC through targeting OTUD7B, TNIP2, and BAD, which were key inhibitors of the NF-κB signaling pathway and proapoptosis protein [36]. However, the effect of the circ_0008193/miR-1180-3p/TRIM62 axis on cell apoptosis was not determined in this study, and this could be another direction of our study.

HK2 and LDHA were direct target glycolytic enzymes of HIF1 [37]. MMP2 and MMP9 expression was in a HIF1-dependent manner [38]. In this present study, we identified TRIM62 as a target of miR-1180-3p associated with HIF1A expression in hypoxic LUAD cells. TRIM62, as a RING finger E3 ubiquitin ligase, has been an adverse prognostic biomarker in different cancers. For example, Lott et al. claimed that TRIM62 was an independent predictor of survival of early-onset breast cancer, cervical cancer, and acute myeloid leukemia [16,17,39]. The physiological mechanism of TRIM62 in cancer is partially by working as a master regulator of EMT and cell polarity [17,40,41]. However, we did not investigate the effect of the circ_0008193/miR-1180-3p/TRIM62 axis on EMT, even though transwell migration and invasion properties have been observed. In lung cancer, TRIM62 loss cooperated with K-ras mutation in promoting lung cancer cell metastasis in vivo [18]. Here, we also noticed a silencing of TRIM62 in LUAD patients and cell lines, and even lower expression of TRIM62 in hypoxia-treated A549 and H1975 cells. Functionally, downregulating TRIM62 was one molecular mechanism of hypoxia-induced cell proliferation, migration, invasion, and Warburg effect. The deletion of TRIM62 was also associated with hypoxia-involved proteins including HIF1A and glucose-related protein 78 in acute myeloid leukemia [39]. Here, we observed that HIF1A upregulation in hypoxia-treated A549 and H1975 cells induced inhibition of TRIM62 expression, and the high level of TRIM62 mediated by circ_0008193 caused HIF1A downregulation. This finding suggested a reciprocal inhibitory effect between HIF1A and TRIM62. However, whether TRIM62 is a novel target of HIF1A remains to be determined. Meanwhile, the relationship between TRIM62 and MMP2, MMP9, HK2, or LDHA before this research was unknown. We declared a negative regulatory effect of TRIM62 on levels of MMP2, MMP9, HK2, and LDHA in LUAD cells under hypoxia in vitro. Its underlying mechanism should be further explored.

Conclusions

Collectively, we demonstrated that circ_0008193 served as a tumor suppressor in LUAD. Overexpression of circ_0008193 suppressed cell proliferation, migration, invasion, and Warburg effect of LUAD cells under hypoxia in vitro and in vivo through promoting TRIM62 via miR-1180-3p. This study suggested a novel circ_0008193/miR-1180-3p/TRIM62 axis underlying the molecular mechanism of hypoxic LUAD.

Conflicts of interest

None.
### Supplementary Data

**A**

![Graph A]

**B**

![Graph B]

**C**

![Graph C]

### Supplementary Figure 1. Expression of hypoxia-inducible factor 1 alpha (HIF1A) in human lung adenocarcinoma (LUAD) cells under hypoxia in vitro. (A–C) Real-time quantitative polymerase chain reaction (RT-qPCR) detected HIF1A messenger ribonucleic acid (mRNA) level in (A) A549 and H1975 cells treated with hypoxia for 48 h or not, and hypoxia-treated A549 and H1975 cells pretreated with empty vector (vector) alone, vector carrying circ_0008193 (circ_0008193) alone or together with (B) miR-1180-3p mimic (miR-1180-3p) or miR-NC mimic (miR-NC), and (C) small interfering (si)RNA against TRIM62 (si-TRIM62) or its negative control (si-NC). * \( P<0.05 \) from three independent experiments.

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