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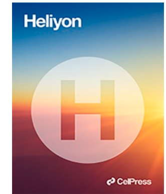

Research article

MiR-210 regulates lung adenocarcinoma by targeting HIF-1α

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A R T I C L E I N F O

Keywords:
miR-210
HIF-1α
VEGF
Lung adenocarcinoma
Overall survival

A B S T R A C T

Object: This study sought to elucidate the role of microRNA-210 (miR-210) in the occurrence and development of lung adenocarcinoma (LUAD).
Methods: The levels of lncRNA miR-210HG and miR-210 in LUAD tissues and corresponding normal tissues were analyzed by real-time quantitative PCR. The expression of the anti-hypoxia factor hypoxia inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) were measured by qRT-PCR and Western blot. The target of miR-210 on HIF-1α was confirmed using TCGA, Western blot and luciferase reporter assay. The regulatory role of miR-210 on HIF-1α and VEGF in LUAD was investigated. The correlation of genes with clinical prognosis was analyzed using bioinformatics methods. The effect of miR-210 on LUAD cells was verified through apoptosis assays.
Results: The expression of miR-210 and miR-210HG was significantly higher in LUAD tissues than in normal tissues. The expression of hypoxia-related indicators HIF-1α and VEGF was also significantly higher in LUAD tissues. MiR-210 suppressed HIF-1α expression by targeting site 113 of HIF-1α, thereby affecting VEGF expression. Overexpression of miR-210 inhibited HIF-1 expression by targeting the 113 site of HIF-1, thereby affecting VEGF expression. Conversely, inhibition of miR-210 resulted in a significant increase in HIF-1α and VEGF expression in LUAD cells. In TCGA-LUAD cohorts, the expression of VEGF-c and VEGF-d genes in LUAD tissues was significantly lower than in normal tissues, while overall survival was worse in LUAD patients with high expression of HIF-1α, VEGF-c and VEGF-d. Apoptosis was significantly lower in H1650 cells after miR-210 inhibition.
Conclusion: This study reveals that miR-210 exerts an inhibitory effect on VEGF expression by down-regulating HIF-1α expression in LUAD. Conversely, inhibition of miR-210 significantly reduced H1650 apoptosis and led to worse patient survival by upregulating HIF-1α and VEGF. These results suggest that miR-210 could serve as a potential therapeutic target for the treatment of LUAD.

1. Introduction

Lung cancer is a prevalent and highly lethal cancer, with a low survival rate of only 19% [1]. According to the American Cancer

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Society's 2020 statistics, lung cancer is the second most common cancer in both males and females, accounting for 13% and 12% of all cancer types, respectively. In China, more than two-thirds of lung cancer patients were diagnosed with lung adenocarcinoma (LUAD) in 2015 [2]. As a predominant subtype of non-small cell lung cancer (NSCLC), LUAD has garnered significant attention in oncology research for its underlying cellular and molecular mechanisms. Despite the abundance of big data, there remains a critical need for in-depth wet lab studies to wncover the complex molecular mechanisms of LUAD, which could enhance our understanding of disease management in clinical settings.

In recent years, mounting evidence has indicated the implication of long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) in tumorigenesis and the development of cancer development [3,4]. MiRNAs, which are small non-coding RNAs (ncRNAs), consisting of 22 nucleotides, interact with target mRNAs to trigger their degradation, while lncRNAs. which are long non-coding RNAs, exhibit more intricate mechanisms [5] such as epigenetic regulation [6]. Hypoxia, a prevailing hallmark of the tumor microenvironment, is associated with the expression of ncRNAs [7]. A former investigation disclosed that 122 out of 7400 lncRNAs showed differential expressed in vascular endothelial cells under hypoxia, with miR-210HG being the most notable, serving as a precursor for hypoxia-inducible miR-210 [8]. Furthermore, hypoxia-inducible factor-1 alpha (HIF-1α), a transcription factor, reacts to hypoxia by stimulating the release of vascular endothelial growth factor (VEGF) for angiogenesis, which is a signal protein that replenishes oxygen supply to tissues. Recent research has identified the significant role of HIF-1α in the transcription of miR-210 [9,10]. It has also been reported that the expression of miR-210 is associated with the prognosis of breast cancer, pancreatic cancer, and NSCLC [11–13]. Despite the crucial research findings provided by the study of miRNA in peripheral blood as a tumor marker for tumor diagnosis and prognosis [14], the implication of miR-210 in the pathogenesis of LUAD has yet to be fully elucidated. Ren et al. [15] identified nine mRNAs that could potentially serve as biomarkers for LUAD, including miR-210. Xie et al. [16] demonstrated that miR-210 can promote the proliferation, migration, and invasion of LUAD by targeting lysyl oxidase-like 4. However, no study has explored the role of miR-210 in the pathogenesis of LUAD at the genetic level in greater depth.

This study intended to characterize the lncRNA-miRNA-mRNA networks in LUAD, to enhance comprehension of the functions of miR-210HG, miR-210 and the target genes in the tumorigenesis. Additionally, we assessed the association between miRNA expression and the overall survival of LUAD patients through survival analysis and prediction, utilizing the TCGA database.

2. Materials and methods

2.1. Patients and tissue samples

From December 2015 to December 2017, one hundred LUAD tissues and one hundred corresponding normal paracancerous tissues were obtained successively from one hundred patients diagnosed with LUAD at the Tumor Hospital Affiliated with Xinjiang Medical University. The Sixth Edition of the TNM Classification of Malignant Tumors (Union for International Cancer Control) was used for TNM staging. All tissue samples were surgical resection specimens. Inclusion criteria were as follows: (1) 18 years of age or older; (2) measurable disease according to Response Evaluation Criteria in Solid Tumors version 1.1; (3) histologic features of adenocarcinoma. Exclusion criteria: patients who had received any form of treatment before surgical resection, including chemotherapeutic treatment, were excluded from the study. The criteria for suspending or terminating the clinical trial were patients who passed away or were lost to follow-up. All patients signed an informed consent form before participating in the study. The collection of tissue samples was approved by the Ethics Committee of Tumor Hospital affiliated with Xinjiang Medical University (No. 2019BC007).

H1650, a non-small cell lung cancer (NSCLC) cell line, was purchased from Cobioer, China with STR document, and cultured in RPMI1640 medium with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Since LUAD is a major subtype of NSCLC, H1650 cells were used here for the experiments.

2.2. QRT-PCR

The total RNA samples from both LUAD tissues and corresponding para-cancerous normal tissues were extracted with TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's instruction. RNA quality was determined using NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and samples with A260/A280 ratio between 1.8 and 2.0 were selected for qRT-PCR. The RNA was subjected to reverse transcription into cDNA after undergoing annealing, extension, and inactivation processes. The cDNA was then diluted with RNase-free water. The expression of miR-210HG, miR-210, HIF-1α and VEGF in the tissue samples were determined using GoTaq 2-Step qRT-PCR System kit in accordance with the protocol provided by the manufacturer. The thermal cycling conditions involved an initial incubation at 96 °C for 5 min, followed by 40 cycles of amplification at 95 °C for 30s and 68 °C for 20s. GAPDH served as the internal reference. $2^{-\Delta\Delta Ct}$ method was applied to analyze the relative expression levels between LUAD tissues and normal tissues. 96 samples were examined in qRT-PCR assay. Primer sequences were as follows: miR-210, forward: 5′-GTGCAGGGTCCGAGGT-3′ and reverse: 3′-TATCTGTGCGTGTGACAGCGGCT-5'; U6, forward: 5′-CTCGCTTCGGCAGCACA-3′ and reverse: 3′-AACGCT-TCACGAATTTGCGT-5′. U6 was used as the internal reference.

2.3. Dual-luciferase reporter assay

To investigate the role of miR-210 in tumorigenesis, the MiR Path DB v1.0 database was applied to identify its targets, which were found to be VEGF and HIF-1α. Target Scan (http://www.targetscan.org/vert_72/) and Pic Tar (https://pictar.mdc-berlin.de/) showed that there were two miR-210 binding sites in the 3′UTR region of HIF-1α mRNA (Fig. S1).

To construct the luciferase reporter plasmid, a 200 base pairs sequence of miR-210 binding sites was amplified from human cDNA. The two binding sites on HIF-1α gene to miR-210 were 113–119 and 250–257, so wild-type HIF-1α 3′UTR (WT) fragment containing the predicted miR-210 binding sites was amplified. The products were amplified by double-digestion PCR and ligated to the psi-CHECK-2 vector (Trangen). Meanwhile, overlapping PCR was used to amplify fragments containing miR-210 mutation site, and the two fragments were ligated into psiCHECK-2 vector. Subsequently, the HIF-1α 3′UTR-WT or HIF-1α 3′UTR-MUT was constructed into the luciferase reporter vector (Shanghai GenePharma Co., Ltd), namely WT, HIF-1α Mut1, HIF-1α Mut2 and Mut1+Mut2, respectively. The primers of mutant HIF-1α 3′UTR were shown as follows: site 113–119 of HIF-1α forward, 5′-CAAACCAGAGAAAGGCAGUGGCU-3′ and reverse, 3′-GUCACACGCCACCCGUCACCGA-5'; site 250–257 of HIF-1α forward, 5′-CAGUGGUGCUCAGGACAGUGGCA-3′ and reverse, 3′-GUCACACGCCACCCGUCACCGA-5′.

The luciferase reporter plasmids (wild-type or mutant) were co-transfected with either miR-210 mimic or NC mimic into HEK-293T cells, with the dual-luciferase reporter vector being used as a negative control. After 48 h of transfection, the luciferase activity was analyzed using a luminometer in accordance with the instructions provided by the Dual-Luciferase Reporter Assay System (Promega).

2.4. Cell culture and transfection

The H1650 cell line, which is derived from human non-small cell lung cancer cells, was cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin and streptomycin (Gibco). The cells were incubated in a humidified incubator at 37 °C with 95% air and 5% CO_2. To manipulate miR-210 expression in these cells, miR-210 mimic, miR-210 inhibitor, or NC were transfected into the cells using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.). The detailed sequences (Shanghai GenePharma Co., Ltd) were as follows: hsa-miR-210-5p mimic, 5′-CUGUGCGUCUGACAGCGGCUGA-3′; hsa-miR-210-5p inhibitor, 5′-UCAGCCGCUGUCACACGCACAG-3'; NC forward, 5′-UUCUCCGAACGUGUCACGUTT-3′; NC reverse, 3′-ACGUGACACGUUCGGAGAATT-5′. After 48 h, the cells were screened for miR-210 expression, and those with stable overexpression or knockdown of miR-210 were used for subsequent experiments.

2.5. Western blot

The protein expression of HIF-1α and VEGF was determined by Western blotting. After 48 h transient transfection, the cells were lysed with RIPA lysis buffer supplemented with Protease Inhibitor Cocktail, and total protein was obtained for subsequent analysis. The concentration of protein was determined using Bradford method, while proteins were resolved on SDS-PAGE and transferred to PVDF membrane for labeling. After blocking the PVDF membrane for 1 h with 5% skim milk powder dissolved in Tris-buffered saline containing 5% Tween-20, the proteins were washed and incubated with VEGF Rabbit pAb (ABclonal, A12303) and HIF1α Rabbit pAb (ABclona, A6265) overnight at 4 °C, respectively. The NcmECL Ultra (P10100, NCM Biotech) was carried out to obtain images.

2.6. Database prediction and TCGA database analysis

Database MiR Path DB v1.0 was employed to forecast the targets of miR-210, whereas the original data from RNA-seq of LUAD were procured from another database named The Cancer Genome Atlas (TCGA) of the United States to authenticate the disparities in gene expression, and scrutinize the correlation between genes and clinical prognosis. A comprehensive total of 542 samples encompassing 59 normal tissues and 483 LUAD tissues were scrutinized for follow-up research. Following the screening of HIF-1, miR-210HG and VEGF gene expression in normal and LUAD tissues, respectively, the correlation between the expression of HIF-1, miR-210HG and VEGF genes and the survival time and overall survival rate of LUAD patients was analyzed.

2.7. Cell apoptosis assay

H1650 cells that were transfected with either miR-210 mimics, miR-210 inhibitors, or NC were seeded at a density of 1×10^6/mL in 6-well plates, with NC-transfected cells serving as controls. After 24 h of incubation, the cells were transferred to 5 mL culture tubes at a concentration of 1×10^6/mL, 100 μL per tube. Next, 5 μL of FITC Annexin V (BD Pharmingen, 51-65874X) and 5 μL of PI (BD Pharmingen, 51-66211E) were added, vortexed and incubated for 15 min in the dark at room temperature. To the mixture, 400 μL of 1 × Annexin V Binding Buffer (BD Pharmingen, 51-66121E) was added, and the sample was analyzed by flow cytometry within 1 h.

2.8. Statistical analysis

The data were analyzed by SPSS Statistics 20.0 (IBM) and R 3.5.3 software. Survival curves were estimated by Kaplan-Meier method. Paired sample *t*-test was used for differential analysis of lncRNA expression in LUAD tissues and normal tissues. Two independent samples *t*-test was applied to the rest of the statistical analysis. The maxstat method was used to determine the cut-off value for classifying LUAD patients into high and low expression groups [17]. Results are expressed as mean ± standard deviation. A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Aberrant expression of hypoxia-inducing genes lncRNA miR-210HG and miR-210 and anti-hypoxia factors HIF-1α and VEGF in lung adenocarcinoma tissues

The qRT-PCR results, as presented in Fig. 1, demonstrated that both miR-210 and its precursor lncRNA miR-210HG were markedly upregulated in LUAD tissues as compared to the normal tissues adjacent to cancer ($P < 0.001$). This suggests that miR-210 may play a crucial role in the development of LUAD. Meanwhile, the expression levels of HIF-1α and VEGF, which are involved in regulating hypoxic environment, were also significantly higher in LUAD tissues compared to normal tissues ($P < 0.001$).

3.2. MiR-210 directly targeted HIF-1α

Based on the miRNA sequence targeting prediction results, 2 potential miR-142-5p binding sites in the HIF-1α 3′UTR were noticed. Thus, HIF-1α WT, HIF-1α Mut1 or HIF-1α Mut2 was constructed into the luciferase gene plasmids. Subsequently, the expression plasmid was co-transfected into H1650 cells with miR-210 mimic or NC mimic. As presented in Fig. 2A, the luciferase activity was significantly reduced in the WT group and HIF-1α Mut2 by miR-210 mimic ($P < 0.01$). The luciferase activity of HIF-1 Mut1 group did not change significantly, while the trend of Mut 1 + Mut 2 group was almost the same as that of Mut 1 group, and there was no significant change, suggesting that site 113–119 of HIF-1α was the actual target of miR-210.

VEGF is transcriptionally regulated by HIF-1α [18]. To further study the relationship between HIF-1α Mut1 and VEGF, miR-210 mimic and extracted plasmid were co-transfected and the mRNA expression of VEGF was detected by qRT-PCR. The results showed that compared with downregulated mRNA expression of VEGF in WT group ($P < 0.001$), VEGF mRNA expression in the HIF-1α Mut1 group was unchanged after miR-210 transfection, indicating that miR-210 overexpression regulated VEGF expression, the target gene of HIF-1α, by inhibiting site 113–119 of HIF-1α (Fig. 2B).

3.3. Effect of miR-210 on the mRNA and protein levels of HIF-1α and VEGF

We then transfected NC, miR-210 mimic and miR-210 inhibitor into H1650 cells to find out the mRNA and protein expression of HIF-1α and VEGF genes regulated by miR-210. Compared with the NC group, the mRNA and protein expression of HIF-1α and VEGF were significantly decreased after transfection with miR-210 mimic ($P < 0.05$); on the contrary, mRNA and protein level expression of HIF-1 and VEGF were significantly upregulated after transfection with miR-210 inhibitors ($P < 0.001$) (Fig. 2C and D).

3.4. Validation of HIF-1α, miR-210HG, VEGF gene expression in lung adenocarcinoma and normal tissues

We further validated the expression of HIF-1α, miR-210HG, and VEGF in TCGA database including 59 normal tissues and 483 LUAD tissues. It should be noted that VEGF had 4 subtypes, namely VEGF-a, VEGF-b, VEGF-c and VEGF-d genes. From Fig. 3A–D, the expression of HIF-1α and miR-210HG genes in LUAD tissues was higher than those in normal tissues, but there was no statistical

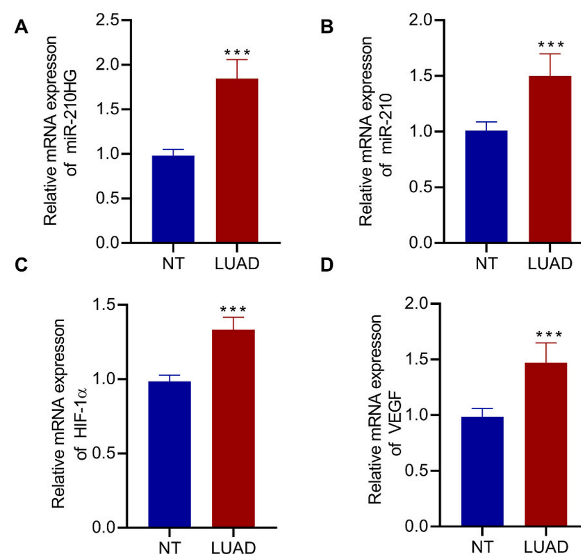

Fig. 1. QPR-PCR was performed to measure the mRNA expression levels of (A) miR-210HG, (B) miR-210, (C) HIF-1α, and (D) VEGF in LUAD and para-cancerous normal tissues. The black bars indicate the para-cancerous normal tissues, and the gray bars indicate the LUAD tissues. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 vs. NT group.

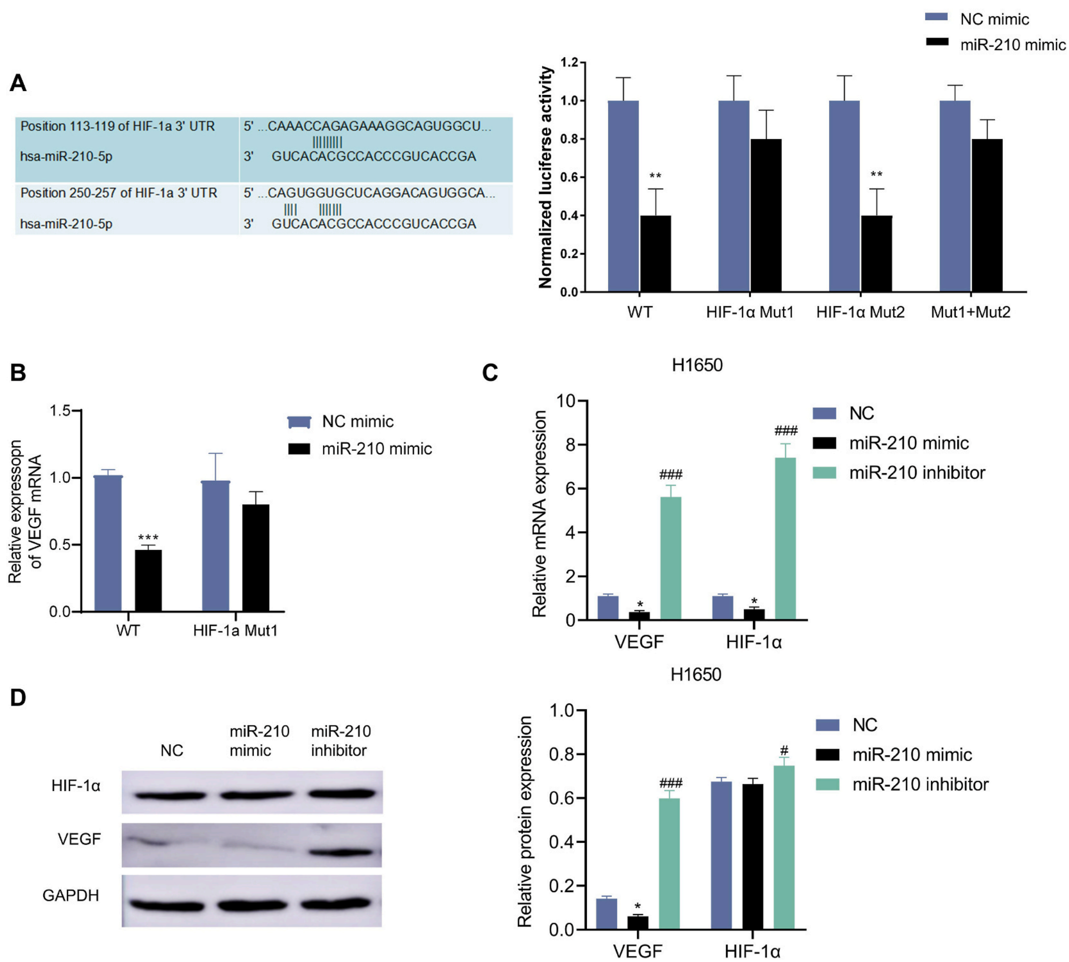

Fig. 2. (A) The dual-luciferase reporter assay was conducted to determine the luciferase activities of HEK-293 cells co-transfected with a luciferase reporter construct containing wild-type or mutant HIF-1α and miR-210 mimic or NC mimic. (B) qRT-PCR was performed to evaluate the effects of miR-210 and HIF-1α on VEGF. In H1650 cells, qRT-PCR and western blot were performed to assess the function of miR-210 on (C) the mRNA expression and (D) the protein expression of HIF-1α and VEGF.

difference, while the expression of VEGF-a and VEGF-b genes in LUAD tissues was almost unchanged compared with normal tissues. In addition, the expression of VEGF-c and VEGF-d genes in LUAD tissues was significantly lower than those in normal tissues (both $P < 0.05$) (Fig. 3E and F).

3.5. Correlation of the gene expression of HIF-1α, miR-210HG and VEGF with overall survival in lung adenocarcinoma patients

We also analyzed the association between overall survival and the expression of HIF-1α, miR-210HG and four VEGF genes. LUAD patients were categorized into high and low expression groups according to the cut-off value determined by Maxstat method. As shown in Fig. 4, LUAD patients with increased expression of HIF-1α, VEGF-c and VEGF-d presented poorer OS compared with the ground expression group ($P < 0.05$), while there was no significant difference between the high and low expression groups of miR 210HG, VEGF-a and VEGF-b.

3.6. Effect of miR-210 on apoptosis in H1650 cells

To examine the effect of miR-210 on LUAD more directly, we examined the apoptosis rate of H1650 cells transfected with miR-210 mimics, miR-210 inhibitors or NC, respectively. The results showed that inhibition of miR-210 significantly inhibited apoptosis of H1650 cells ($P < 0.001$), but the apoptosis rate of H1650 transfected with miR-210 did not change significantly (Fig. 5A–D).

4. Discussion

LUAD is one of the subtypes of NSCLC, accounting for 40% of cases. With the increase in mortality, more and more researchers tend

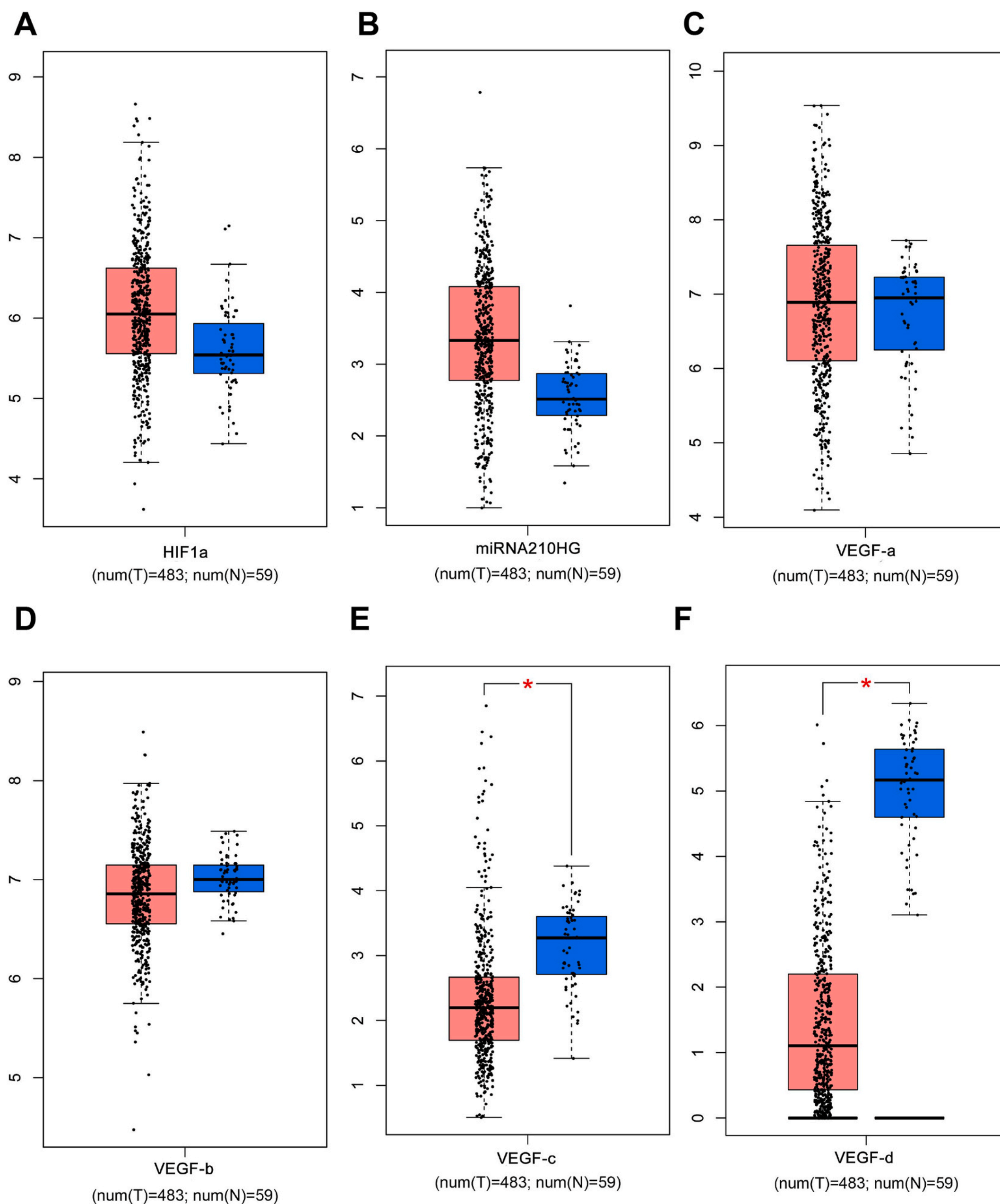

Fig. 3. Validation of miR-210, miR-210HG, HIF-1α and VEGF gene expression in LUAD tissues and normal tissues using the TCGA database. (A): HIF-1α; (B): miR-210HG; (C-F): VEGF a-d genes. The red column represents the group of LUAD tissues, while the blue column represents the group of normal tissues. The horizontal axis of this figure represents the HIF-1α, miR-210HG, miR-210, and VEGF genes in each group, and the vertical axis represents the relative expression level. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

to identify the role of lncRNAs, miRNAs, and the mutual regulation of lncRNAs and miRNAs in cancer development. As an important factor in post-transcriptional regulation, miRNA activity could be regulated by lncRNA through "sponge" adsorption [19,20]. This kind of lncRNA was also named Competitive endogenous RNA (ceRNA). As a ceRNA, lncRNA competitively binds to miRNA, thereby regulating the protein level of coding genes and participating in regulating the biological behavior of cells [21,22]. Many lncRNAs can function as "sponges" for miRNAs due to the similar structures between lncRNAs and mRNAs. In addition, the patterns of gene expression regulation are more diverse and extensive, and will not be disturbed by translation [23]. lncRNA-miRNA-mRNA is a new regulation mode that supplements the traditional miRNA-mRNA regulation mode, which has a significant effect on the progression and pathogenesis of many tumors, including LUAD [3,4,24].

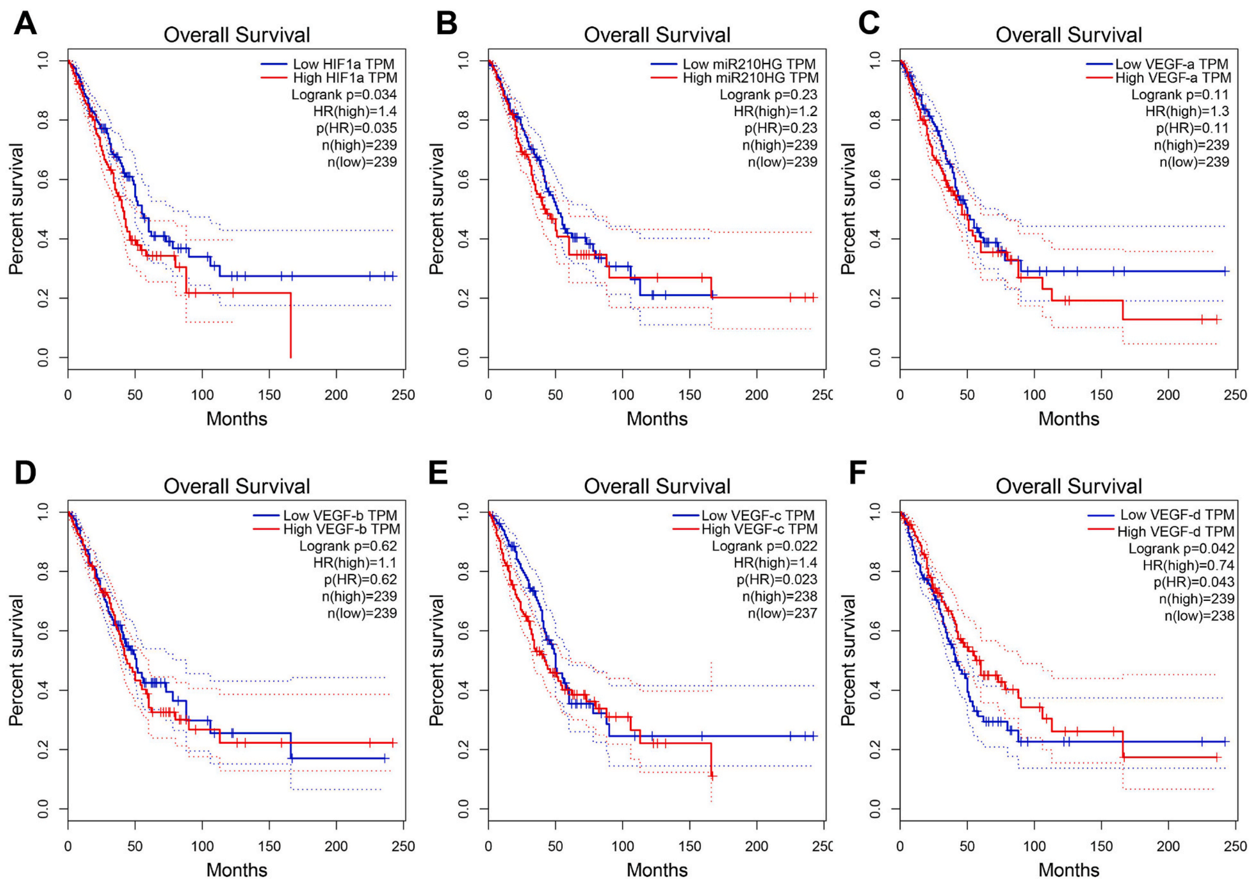

Fig. 4. The correlation between the expression levels of HIF-1α, miR-210HG and VEGF genes and overall survival in LUAD patients. (A) HIF-1α; (B) miR-210HG, and (C–F) VEGF a–d. The log-rank test was used to calculate the significant differences between the high and low expression groups.

Accumulating studies have shown that miR-210 is a stable target for HIF, and its overexpression could be stimulated by cell migration, capillary-liked formation and VEGF. VEGF can be upregulated by HIF-1α and plays an important role in tumor angiogenesis [25,26]. The results of this study showed that the expression levels of miR-210HG and miR-210, in LUAD tissues were higher than those in normal tissues, suggesting that miR-210 is involved in the occurrence and development of LUAD. Of note, the expression of HIF-1α and VEGF, which can regulate hypoxia and restore tissue oxygenation, was also higher in LUAD tissues than in normal tissues. Then, bioinformatics prediction was carried out to find potential target binding sequences of HIF-1α and miR-210. The results of dual-luciferase reporter assay revealed that miR-210 directly targeted HIF-1α. Du et al. [27] found that miR-210-3p regulates HIF-1α and p53 activity by directly targeting GPD1L and CYGB, enhancing aerobic glycolysis. miR-210 is in turn a direct transcriptional target of HIF-1α, indicating a positive feedback loop between miR-210 and HIF-1α [28]. However, the regulatory effect of miR-210 on VEGF has yet to be explored.

In the present research, compared with the control group, the mRNA and protein expression of HIF-1α and VEGF genes were significantly increased after the addition of miR-210 inhibitor, which was reversed after miR-210 mimic transfection. These results further confirmed that miR-210 targeted HIF-1α and VEGF genes through site 113 of HIF-1α (Fig. 6). Likewise, Chen et al. have suggested that in NSCLC tissues, the positive rate of HIF-1α was significantly higher than that of benign lung lesions [29]. Our study also clarified the possible molecular mechanism of miR-210, and how miR-210 has an impact on the tumor cells under hypoxia. In fact, under the hypoxic microenvironment, HIF-1α can regulate gene transcription and induce gene expression of tumor cells through a variety of mechanisms downstream, allowing the increased resistance of tumor cells for greater proliferation, metastasis and invasion capabilities [30,31]. Therefore, miR-210 may be a potential novel marker for the early detection and diagnosis of LUAD.

Additionally, a study conducted by Wang et al. pointed out the upregulated miR-210HG in cervical cancer tissues through microarray analysis. The inhibition of miR-210HG significantly suppressed the proliferation, invasion, and epithelial-mesenchymal transition processes, and reduced tumor growth in vivo. Mechanistically, miR-210HG might serve as a ceRNA of miR-503-5p to relieve the inhibitory effect of miR-503-5p on TRAF4 expression in cervical cancer cells [32]. miR-210HG, a transcriptional precursor of hypoxia-inducible marker miR-210, was differentially expressed in hypoxic vascular endothelial cells [8]. However, the results of our research did not show a clear targeting relation between miR-210HG and miR-210, it was unclear whether lncRNA-miR-210HG served as a ceRNA in LUAD.

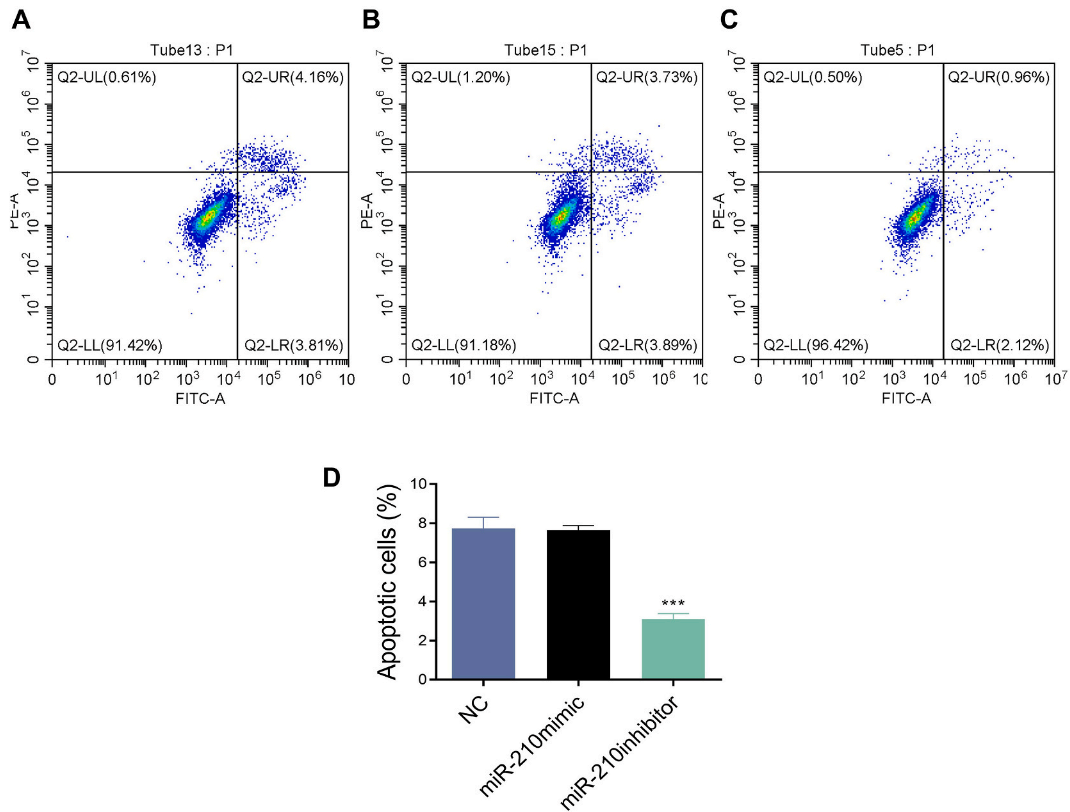

Fig. 5. The effect of miR-210 on apoptosis in H1650 cells. Flow cytometry was performed to detect apoptosis in H1650 cells transfected with NC (A), miR-210 mimic (B) or miR-210 inhibitor (C). The statistical analysis results were presented in the bar graph (D). ***P < 0.001 vs. NT group.

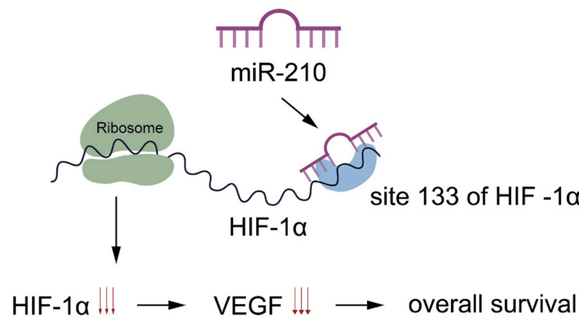

Fig. 6. The functional model of miR-210 regulation of HIF-1. MiR-210 inhibits the expression of HIF-1α by targeting the 113rd site of HIF-1α, thereby reducing the expression of VEGF and increasing the survival rate of patients with lung adenocarcinoma.

Moreover, numerous studies have confirmed that the up-regulation of miR-210 expression is associated with poor prognosis in multiple tumors [33,34]. The relationship of the high miR-210 expression with shorter overall survival, metastasis-free survival or distant relapse-free survival, and disease specific survival was observed [35]. Xie et al. found that miR-210 promotes the proliferation, migration and invasion of LUAD by targeting lysyl oxidase-like 4 [16]. Several studies [15,36,37] have shown that miR-210 is a potential prognostic marker for LUAD. And the results of the present study suggest that the role of miR in LUAD development may be more important than we discovered. VEGF is known to be involved in the occurrence, development, and metastasis of NSCLC, which could be considered as an indicator to predict the malignancy and prognosis of patients [38]. VEGF-c expression is associated with poor prognosis for patients with stage I NSCLC [39], while VEGF-a is the most specific and prominent angiogenic factor among all VEGF family members, which can stimulate the proliferation of vascular endothelial cells and increase the vascular permeability [40]. In the present study, we found the expression of HIF-1α, miR-210HG, VEGF-a, and VEGF-b genes in LUAD tissues were higher than those in normal tissues, however, the expression of VEGF-c and VEGF-d genes in LUAD tissues were lower than those in normal tissues. In terms of prognosis, LUAD patients with higher expression of HIF-1α, VEGF-a, VEGF-c, and VEGF-d had a worse prognosis than the lower

expression group, while patients with lower expression of miR-210HG and VEGF-b had a worse prognosis. In addition, apoptosis assays showed that inhibition of miR-210 inhibited apoptosis in H1650 cells. We know that inhibition of miR-210 can upregulate the expression of HIF-1α and VEGF, and patients with high expression of HIF-1α and VEGF have a poorer prognosis. Therefore, we hypothesize that inhibition of miR-210 upregulated HIF-1α and VEGF expression leads to poorer patient survival by inhibiting apoptosis in LUAD cells.

In addition to different tumor types and different signal transduction pathways, miR-210 has many target genes and different functions, and its effects on tumor cells might be the comprehensive effect of target genes. The role of miR-210 in tumorigenesis and cancer development could provide new ideas for tumor treatment. Xie et al. confirmed that LOXL4 served as a downstream target of miR-210, and miR-210 promoted the progression of lung cancer by targeting LOXL4 [16]. Lai et al. [41] measured the plasma miR-210 content of glioma patients and healthy controls and found that miR-210 level of glioma patients was 7 times higher than that of the controls. The survival analysis of that study showed that high expression of miR-210 in patients with glioma was related to its poor prognosis, indicating miR-210 could be a potential biomarker for the detection and prognosis prediction of glioma. Therefore, it is recommended to detect the miR-210 content and its stability in the blood of patients with LUAD, which may provide a new approach to the diagnosis and treatment of patients with LUAD.

5. Conclusion

This study suggests that the targets of miR-210 are HIF-1α and VEGF, and miR-210 can suppress VEGF expression by downregulating HIF-1α expression in LUAD. Besides, HIF-1α, VEGF-c and VEGF-d expression is closely associated with the overall survival of LUAD patients and provides great help in predicting the prognosis of LUAD patients. This also demonstrates the potential contribution of IncRNA-miRNA-mRNA interaction to the pathogenesis and development of LUAD. However, this experiment only analyzed the prognosis of LUAD patients and the effect of miR-210 on H1650 cell apoptosis, and the study of the mechanism of miR-210 regulation of lung adenocarcinoma was not comprehensive. Subsequently, a systematic study of the mechanism of miR-210 regulation of lung adenocarcinoma will be conducted, and the pathogenesis of LUAD will be explored by IncRNA-miRNA-mRNA interaction.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Tumor Hospital affiliated to Xinjiang Medical University (No. 2019BC007). Signed informed consents were obtained from all patients.

Declarations

Author contribution statement

Guolei Cao; Qin Luo: Conceived and designed the experiments; Wrote the paper.
Peiwen Fan: Performed the experiments.
Ronghui Ma; Qinghe Wang: Analyzed and interpreted the data.
Lili He; Haiwen Niu: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

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

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Abbreviations

LUAD lung adenocarcinoma
NSCLC non-small cell lung cancer
lncRNAs long non-coding RNAs
miRNAs microRNAs

ncRNAs non-coding RNAs
HIF-1α hypoxia-inducible factor-1 alpha
VEGF vascular endothelial growth factor
WT wild-type
MUT mutation
DMEM Dulbecco's modified Eagle medium
TCGA The Cancer Genome Atlas
ceRNA Competitive endogenous RNA

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16079.

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