LncRNA OGFRP1 acts as an oncogene in NSCLC via miR-4640-5p/eIF5A axis

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Abstract

Background

Long noncoding RNAs (lncRNAs) OGFRP1 is up-regulated in endometrial cancer and cervical carcinoma, and OGFRP1 suppression inhibits the malignant behaviour of cancer cells. However, the role of OGFRP1 in non-small-cell lung cancer (NSCLC) have not been investigated. Here, we evaluated the expression pattern, biological function and potential mechanism of OGFRP1 in NSCLC.

Methods

We screened the siRNA (siOGFRP1) to down-regulate the expression of OGFRP1 in A549 and H1299 cells. The biological function of A549 and H1299 cells were examined by CCK8, wound healing and transwell assays. The molecular mechanism of OGFRP1 was further explored.

Results

siOGFRP1 significantly inhibited the cell proliferation, migration and invasion of A549 and H1299 cells. In addition, the expression of EMT-related and apoptosis-related proteins was changed by siOGFRP1 transfection. MiR-4640-5p could directly bind to the 3' UTR region of eIF5A1. Moreover, OGFRP1 bound to miR-4640-5p through the same binding site, which facilitated the expression of eIF5A1. eIF5A1 overexpression rescued cell proliferation, migration and invasion inhibition induced by OGFRP1 down-regulation and miR-4640-5p up-regulation in A549 and H1299 cells.

Conclusions

Taken together, we demonstrated that down-regulation of OGFRP1 inhibited the progression of NSCLC through miR-4640-5p/eIF5A1 axis.

Introduction

Lung cancer is a serious life-threatening disease, leading to 27% of cancer related deaths.[1] Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases.[1, 2] Although therapeutic technologies against NSCLC evolved very rapidly in the past decades, the survival rate of NSCLC remains still low, with ~ 13% (all stages combined) in 5 years.[3] In recent years, with the development of sequencing technology, great improvements have been made in the cost and speed of sequencing. Combined with the development of tumor biology, personalized therapy, which is characterized by gene diagnosis and molecular targeted therapy, has become a promising treatment for NSCLC.[4–6] It is increasingly important to find new molecule targets and identify related action mechanisms for early diagnosis and treatment of NSCLC.

Long noncoding RNAs (lncRNAs) are a category of RNAs longer than 200 bp without protein coding activity.[7, 8] Currently, thousands of lncRNAs have been identified by ENCODE project and GENCODE annotation. However, the corresponding functional annotations of lncRNAs are extremely insufficient, which is partly due to their low expression, high tissue specificity and narrow time frames.[9–15] However, the current studies suggest that lncRNAs are involved in nearly all biological processes, including cancer cell proliferation, apoptosis, migration and invasion through chromatin remodeling and histone modification, epigenetic modification or sponge effect.[16–19] It has been reported that several lncRNAs are important regulators in the progression of NSCLC. For example, lncRNA-PAGBC promoted cell proliferation and metastasis of human gallbladder cancer (GBC) *in vitro* and *in vivo* by sponging tumor suppressive microRNAs miR-133b and miR-511.[16] lncRNA ANRIL functions as a oncogene by interaction with c-Myc in NSCLC.[20] lncRNA FEZF1-AS1 promoted tumor progression by inhibiting E-cadherin and modifying WNT pathway in NSCLC.[21]

Homo sapiens opioid growth factor receptor pseudogene 1 (OGFRP1), with 1201 nucleotides in length, is a recently identified lncRNA located on chromosome 22q13.2. OGFRP1 is found to be up-regulated in endometrial cancer[22] and cervical carcinoma.[23] Furthermore, OGFRP1 suppression inhibits the malignant behaviour (inhibits cell viability, promotes apoptosis, and suppresses cell migration and invasion) of the endometrial cancer cells (Ishikawa),[22] hepatocellular carcinoma cells (Hep3B),[24] cervical carcinoma cells (C33A and SiHa)[23], gestational choriocarcinoma cells (JEG3)[25] and human coronary artery endothelial cells (HCAECs).[26] However, the expression pattern, biological function and potential mechanism of OGFRP1 in NSCLC have not been investigated. Although, Ding and Liu analyze the RNA-seq data of 551 lung adenocarcinoma (LUAD) patients downloaded from The Cancer Genome Atlas (TCGA), and find that OGFRP1 as an interesting factor involves in the LUAD.[27]

In this study, we used siOGFRP1 to investigate the role of OGFRP1 in NSCLC. Then we examined the changes of miR-4640-5p/eIF5A1 axis to explain the action mechanism of OGFRP1.

Materials And Methods

Cell culture and transfection

Human non-small cell lung cancer cell lines (A549 and H1299) were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China) and cultured in RPMI-1640 medium (Gibco, USA) supplemented with 10% FBS (Hyclone, USA), 100 U/ml penicillin and 0.1 mg/ml streptomycin at 37°C with 5% CO₂ atmosphere. Lipofectamine2000 liposome was used to transfect siRNA or plasmid into cells following the instructions. siRNAs targeting to OGFRP1 (siOGFRP1) were designed and synthesized (RiboBio, Guangzhou, China). miR-4640-5p mimic was purchased from RiboBio (Guangzhou, China). The cDNA of eIF5A1 was synthesized by GENEWIZ and cloned into the pcDNA3.1 expression vector (GenePharma, Shanghai, China).

qRT-PCR

Total RNA was extracted by using TRIzol (Invitrogen) according to the manufacturers' instructions. The cDNA was formed by using EasyScript™ Reverse Transcriptase (TransGen Biotech Co., Ltd., Beijing, China). The mRNA expression was further detected by using an FTC-300 Real-Time Quantitative Thermal Cycler (Funglyn Biotech Inc., Shanghai, China). GAPDH was used as an internal reference.

CCK8 assay

After 24 h of transfection, the cells were digested, resuspended and counted. 1000 cells were planted in each well of a 96-well plate. Cell viability was measured every 24 hours. For testing, 10 µl of CCK8 reagent was added to each well of the 96-well plate, and incubated at 37 °C for 2 h. Then the OD value at 450 nm was measured to draw the proliferation curve.

Wound healing migration assay

After transfection for 24 h, cells were scratched by a sterile pipettes tip and washed by PBS to eliminate suspended cells. Subsequently cells were cultured in fresh medium and photographed at 0 h and 24 h.

Transwell assay

The transwell chamber was coated with Matrigel. The cells that had been transfected for 24 hours were prepared into a cell suspension with serum-free medium. 100 µl of cell suspension containing 1×10^4 cells was added to the upper chamber, and 600 µl of medium containing 10% FBS was added to the lower chamber. After culturing for 24 hours, the residual cells on the upper chamber were removed and washed with PBS. Then the cells on the lower surface of the chamber were fixed with paraformaldehyde for 15 min and stained with 0.1% crystal violet for 5 min. After washed with PBS, the cells were photographed and counted under a microscope.

Western blot

After 48 h of transfection, the total proteins of the cells were extracted using RIPA buffer. 20 µg of proteins were taken for SDS-PAGE electrophoresis, and then electrotransferred onto a PVDF membrane. The membrane was blocked with 5% non-fat milk for 1 h, incubated with the specific primary antibody at 4 °C overnight and incubated with the second antibody for 1 h at room temperature. ECL development was performed after washing the membrane through TBST.

Luciferase reporter assay

The complete 3'UTR of human eIF5A1 mRNA containing the putative or mutated miR-4640-5p binding site, and the wild or mutated full-length sequence of OGFRP1 were amplified and cloned into the psiCHECK2 vector (Promega). According to the manufacturer's guidelines, Lipofectamine 2000 was used to co-transfect psiCHECK2 recombinant vector and miR-4640-5p mimic or miR-NC into A549 cells. The relative activity of luciferase was measured using the Dual-Luciferase Reporter Assay System (Promega) and the Infinite M200 PRO microplate reader (Tecan, Shanghai, China).

Statistical analysis

All data were statistically analyzed using SPSS software version 22.0 (IBM Corp., Armonk, NY). All results were expressed as mean $\pm$ standard deviation. The difference between groups was calculated using the Student's t-test or one-way ANOVA. $P < 0.05$ was considered statistically significant.

Results

OGFRP1 is high expressed in NSCLC patients and may be a prognostic marker

We firstly investigated the expression level of OGFRP1 in LUAD and normal tissues. All data comes from GEPIA. As shown in Fig. 1A, the expression of OGFRP1 in LUAD patients was elevated by 2 folds compared to that in normal controls ($P < 0.05$). The basic expression pattern implied that OGFRP1 might play positive role in NSCLC progression. The correlation between the expression of OGFRP1 and survival of LUAD patients analyzed on GEPIA was shown in Fig. 1B. The result illustrated a lower survival rate in LUAD patients with a high OGFRP1 expression (HR = 1.6, $P = 0.002$). The above results suggested that OGFRP1 was involved in the tumorigenesis of NSCLC and could be a potential therapeutic target or poor-prognosis marker for NSCLC treatment.

Down-regulation of OGFRP1 inhibits cell proliferation, migration and invasion in NSCLC

After preliminary investigation of the correlation between OGFRP1 expression and NSCLC tumorigenesis and prognosis, we synthesized 3 siRNAs with different target sites to inhibit the expression of OGFRP1. As shown in Fig. 1C, the most powerful siRNA1 was used for the subsequent experiments. We first studied the effect of siOGFRP1 on the proliferation of NSCLC cells by using CCK8 proliferation assay. As shown in Fig. 1D and E, the OD value of siOGFRP1 group was significantly reduced than that in NC group. Cell migration was investigated by wound healing assay. The result was shown in Fig. 2A, which indicated that compared to NC group, the wound width in siOGFRP1 group remained relatively greater ($P < 0.05$). The relative migrated area at 24 h (Fig. 2A) also suggested a significant difference between NC and siOGFRP1. Subsequently, cell invasion was investigated by in vitro Matrigel invasion assay. The result shown in Fig. 2B indicated that the number of invasive NSCLC cells (crystal violet stained) was much lower than that in groups of NC ($P < 0.05$). Furthermore, expression of E-cadherin was up-regulated, while N-cadherin, Vimentin, Snail1 and Snail2 were down-regulated in siOGFRP1 group (Fig. 2C). In addition, western blotting analysis revealed increased expression of Bax and cleaved caspase 3, alongside decreased expression of Bcl2 in siOGFRP1 group (Fig. 2D). These results revealed that OGFRP1 played an important role in NSCLC cell proliferation, migration, invasion and apoptosis.

OGFRP1 acts as a ceRNA targeting eIF5A1 via miR-4640-5p

For mechanism research, the algorithm predicted that OGFRP1 could act as a ceRNA to target eIF5A1 via miR-4640-5p and luciferase assay validated this (Fig. 3A and B). As shown in Fig. 3A, A549 cells co-transfected with miR-4640-5p mimic and OGFRP1-WT showed less luciferase activity than the other

groups. In parallel, it was observed that A549 cells co-transfected with miR-4640-5p mimic and eIF5A1-WT revealed less luciferase activity than the other groups (Fig. 3B).

OGFRP1 exerts its role through regulating miR-4640-5p/eIF5A1 axis

Finally, to test whether OGFRP1 exerted its role through regulating miR-4640-5p/eIF5A1 axis, cells were transfected with pcDNA3.1-eIF5A1 overexpression plasmid (eIF5A1), pcDNA3.1-eIF5A1 plasmid and siOGFRP1 (eIF5A1 + siOGFRP1), pcDNA3.1-eIF5A1 plasmid and miR-4640-5p mimic (eIF5A1 + miRNA mimic), respectively. We tested the expression levels of OGFRP1, miR-4640-5p and eIF5A1 in the three groups of cells (Fig. 3C). Furthermore, eIF5A1 overexpression promoted cell proliferation, migration and invasion of A549 and H1299 cells (Fig. 4A-C). And, eIF5A1 overexpression changed the expression of Epithelial-mesenchymal transition (EMT)-related and apoptosis-related proteins (Fig. 4D and E). In addition, eIF5A1 overexpression rescued cell proliferation, migration and invasion inhibition induced by OGFRP1 down-regulation and miR-4640-5p up-regulation in A549 and H1299 cells (Fig. 4). These results showed that OGFRP1 may regulate NSCLC cells process through miR-4640-5p/eIF5A1 axis.

Discussion

As the development of RNA sequencing technology, thousands of lncRNAs were identified, which accounted for most of genome transcripts and regulated a large range of cell processes. [12, 28–30] With respect to cancer, lncRNAs were found to play important roles in cancer progression *in vitro* and *in vivo*. [16, 31–34] However, lncRNAs having been functionally annotated only occupied a small part of total lncRNAs. More researches were needed on the function of lncRNAs, especially those with important prognostic and therapeutic values.

In this study, we aimed to determine the functions of OGFRP1 in NSCLC and the underlying mechanisms. On GEPIA we found that expression of OGFRP1 was up-regulated in LUAD and negatively correlated with the survival rate of patients, which suggested that OGFRP1 might be a prognostic biomarker or therapeutic target. Then we screened a most effective siRNA (siOGFRP1) from 3 candidates to knock down the expression of OGFRP1 and examined the effects on A549 and H1299 cells. Through CCK8 assay we found that siOGFRP1 could significantly inhibit A549 and H1299 cell proliferation. Cell migration and invasion were also inhibited by siOGFRP1 in wound healing assay and transwell invasion assay. In addition, the expression of EMT-related and apoptosis-related proteins was changed by siOGFRP1 transfection. These data revealed the oncogene function of OGFRP1 in NSCLC, which was consistent with the findings in endometrial cancer,[22] hepatocellular carcinoma,[24] gestational choriocarcinoma cells (JEG3)[25] and cervical carcinoma cells.[23]

Eukaryotic translation initiation factor 5A (eIF5A) is an 18-kDa protein that participates in mRNA-related functions, such as transcription,[35, 36] mRNA turnover[37] and nucleoplasmic transport,[38] plays a role in the initiation and extension of protein synthesis,[39, 40] which is essential for cell proliferation. Vertebrates carry two genes, which encode two highly homologous eIF5A subtypes, namely eIF5A1 and eIF5A2.[41] According to reports, eIF5A1 is highly expressed in a variety of tumors, which is associated

with poor clinical features and prognosis, including lung adenocarcinoma.[40] In lung tumor tissues, eIF5A1 is observed in both the cytoplasm and the nucleus.[40] In the present study, we found that OGFRP1 could act as a ceRNA to target eIF5A1 via miR-4640-5p using luciferase assay. The regulation mechanism of eIF5A1 gene expression has not been fully determined. In lung cancer, increased expression of eIF5A1 protein is associated with oncogenic mutations of *K-ras* at codons 12 and 13,[40] which indicates that the *K-Ras* signaling pathway induces eIF5A expression. Treatment of Bcr-Abl⁺ K562 cells with imatinib (a drug that inhibits Abl tyrosine kinase) can reduce the levels of eIF5A1 protein and mRNA.[42] This finding indicates that eIF5A1 may also be induced by the Bcr-Abl oncogene. Considering the incomplete correlation between eIF5A1 mRNA and protein levels, this may also mean that there is translation control or other post-transcriptional regulatory mechanisms. A mechanism based on E3 ubiquitin ligase CHIP/Stub1 to induce protein degradation has been reported.[43] In addition to oncogene-driven transcription and post-transcriptional regulation, our study reported the epigenetic regulation of eIF5A by ceRNA for the first time.

Conclusion

In conclusion, we found that OGFRP1 might be a prognostic biomarker and down-regulation of OGFRP1 inhibited progression of NSCLC by regulating eIF5A1 expression. Our research suggested that OGFRP1 may be a potential molecular target for NSCLC treatment in the future.

Declarations

Authors' contributions

- 1) Conception and design, acquisition of data, or analysis and interpretation of data: All authors.
- 2) Drafting the article or revising it critically for important intellectual content: All authors.
- 3) Final approval of the version to be published: All authors.
- 4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: Xuewei Zhuang.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data supporting the conclusions of this paper are included within the manuscript.

Consent for publication

All the authors agree to the publication clause.

Ethics approval and consent to participate

Not applicable.

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Figures

Figure 1

OGFRP1 is high-expressed in LUAD and represents a worse prognosis. (A) The boxplot of OGFRP1 transcriptional expression in LUAD and controls. The red and gray boxes represent LUAD and normal tissues respectively. The y-axis indicated the log2-transformed gene expression level; (B) Survival curves of LUAD patients with different OGFRP1 expression. The result derived from GEPIA (<http://gepia.cancer-pku.cn/>), which was based on the database of TCGA and GTEx. (C) Interference efficiency of 3 alternative siRNAs was detected by Fluorescence Quantitative PCR. The proliferation of A549 (D) and H1299 (E) cells transfected with siOGFRP1 was measured by CCK8 assay. * represented $P < 0.05$. Abbreviations: lung adenocarcinoma, LUAD; Gene Expression Profiling Interactive Analysis, GEPIA; the Cancer Genome Atlas, TCGA; Genotype-Tissue Expression, GTEx.

Figure 2

Down-regulation of OGFRP1 inhibits migration and invasion of NSCLC cells. (A) Cell migration was examined by wound healing assay. The images were taken at 0 h and 24 h after wound formed. (B) Images of invasive cells in transwell assay. The expression of EMT associated proteins (C) and apoptosis associated proteins (D) was detected by western blot and normalized to GAPDH. * represented $P < 0.05$.

Figure 3

OGFRP1 acts as a ceRNA targeting eIF5A via miR-4640-5p. (A) Upper line: The sequences of miR-4640-5p, wide type of OGFRP1 (WT) and mutated OGFRP1 (Mut). Lower line: The expression levels of luciferase of A549 cells transfected with wild-type (WT) or mutated (Mut) OGFRP1 reporters plus miR-4640-5p mimic or miR-NC were determined. (B) Upper line: The sequences of miR-4640-5p, wide type of eIF5A (WT) and mutated eIF5A (Mut). Lower line: The expression levels of luciferase of A549 cells transfected with wild-type (WT) or mutated (Mut) eIF5A reporters plus miR-4640-5p mimic or miR-NC were determined. (C) Cells were transfected with pcDNA3.1-eIF5A1 overexpression plasmid (eIF5A1), pcDNA3.1-eIF5A1 plasmid and siOGFRP1 (eIF5A1+siOGFRP1), pcDNA3.1-eIF5A1 plasmid and miR-4640-5p mimic (eIF5A1+miRNA

mimic), respectively. The expression levels of OGFRP1, miR-4640-5p and eIF5A1 were detected by qRT-PCR. * represented $P < 0.05$.

Figure 4

OGFRP1 exerts its role through regulating miR-4640-5p/eIF5A1 axis. Cells were transfected with pcDNA3.1-eIF5A1 overexpression plasmid (eIF5A1), pcDNA3.1-eIF5A1 plasmid and siOGFRP1 (eIF5A1+siOGFRP1), pcDNA3.1-eIF5A1 plasmid and miR-4640-5p mimic (eIF5A1+miRNA mimic), respectively. (A) The proliferation of A549 and H1299 cells was measured by CCK8 assay. (B) Cell migration was examined by wound healing assay. The images were taken at 0 h and 24 h after wound formed. (C) Images of invasive cells in transwell assay. The expression of EMT associated proteins (d) and apoptosis associated proteins (E) was detected by western blot and normalized to GAPDH. * represented $P < 0.05$.