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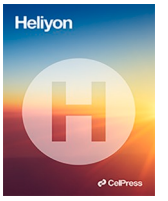

Research article

Up-regulated ORC1 promotes lung adenocarcinoma by inhibiting ferroptosis via SLC7A11 dependent pathway

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ABSTRACT

Background: Lung adenocarcinoma (LUAD) is a pulmonary malignant disease that poses a high risk of mortality and morbidity. Previous study indicated that ORC1 plays an oncogenic function. However, the precise regulatory function that ORC1 serves in the progression of LUAD is still not clearly known.

Methods: Bioinformatics analyses were performed using TCGA and GEO datasets. The human LUAD cell line NCIH1355, NCIH1568 as well as BEAS-2B cell line (human normal lung epithelial cell) were utilized for in vitro study. LUAD cell proliferation were determined via CCK-8 assays and RT-qPCR for ki-67. The relation of ORC1 and SLC7A11 was detected by Western blot and qPCR with or without sh-RNA. The expression level ACSL4, the biomarker of ferroptosis, were detected using RT-qPCR.

Results: ORC1 and SLC7A11 exhibit high expression levels in both LUAD patients and cell lines, and are strongly associated with poor prognosis. In vitro experiments demonstrate that ORC1 and SLC7A11 promote proliferation of LUAD cell lines while inhibiting gefitinib-induced ferroptosis. Additionally, the function of ORC1 in LUAD cells is dependent on SLC7A11.

Conclusion: ORC1 promotes LUAD cell proliferation and inhibits ferroptosis in a SLC7A11-dependent manner. This implies that ORC1 could potentially serve as a useful diagnosis biomarker and treatment target.

1. Introduction

Lung cancer is a significant and deadly cancer globally [1]. The bulk of cases (85 %) are categorized as non-small cell lung cancer (NSCLC) [2], with lung adenocarcinoma (LUAD) being the most frequently occurring subtype, often associated with poor prognosis and high mortality rate[[3,4]]. Despite significant progress made in radiotherapy, surgical resection, targeted treatment, chemotherapy, immunotherapy, traditional Chinese medicine treatment, and other therapies, the efficacies of these treatments are still limited[[5,6]]. The mean 5-year survival rate among LUAD patients still falls beneath 10 % [7]. Consequently, further research is necessary to advance our understanding of the mechanisms underpinning the prognosis of LUAD, and to develop more effective

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Abbreviations

LUAD	Lung Adenocarcinoma
NSCLC	Non-Small Cell Lung Cancer
ORC1	Origin Recognition Complex 1
SLC7A11	Solute Carrier Family 7 Member 11
GSH	Glutathione
TCGA	The Cancer Genome Atlas
AUC	Area Under the Curve
OS	Overall Survival
CCK8	Cell Counting Kit-8
RT-qPCR	Reverse Transcription Quantitative Polymerase Chain Reaction
PVDF	Polyvinylidene Fluoride

therapies to improve patient outcomes.

In eukaryotes, the initial stage of DNA replication includes the origin-recognition complex (ORC), of which ORC1 has a significant role in DNA replication and organ development[[8,9]]. ORC1 is a functional protein that plays a critical role in regulating cellular processes, including apoptosis, proliferation, and cell cycle [10]. Recently years, increasing evidence has shown that ORC1 dysregulation is connected to the tumorigenesis of various human cancers, such as tongue squamous cell carcinoma [11], cervical cancer [12], and triple-negative breast cancer [13]. This suggests that ORC1 could serve as a regulatory protein in human malignancies. Additionally, ORC1 may be involved in migration, apoptosis, cell proliferation, invasion, and other biological processes [14]. Hence, the study of ORC1 molecular mechanism is conducive to understand the occurrence as well as the development of LUAD and finding potential therapeutic targets for LUAD.

Ferroptosis is a programmed cell death mechanism, and researches indicated that dysfunctional ferroptosis may play a role in the development of a series of diseases, including cancer[[15,16]]. This dysfunction occurs through biological processes such as oxidative stress, lipid metabolism, iron metabolism, and glutathione (GSH) [[17,18]]. One of the key regulators of ferroptosis is acyl-CoA synthetase long-chain family member 4 (ACSL4), an enzyme involved in the biosynthesis of polyunsaturated fatty acid (PUFA)--containing phospholipids, which are essential for the propagation of lipid peroxidation and subsequent ferroptotic cell death [33]. Dysregulation of ACSL4 has been implicated in promoting or inhibiting ferroptosis in different cellular contexts, making it an important player in the regulation of lipid metabolism and cell death pathways [19]. Furthermore, recent studies[[19,20]] have highlighted the role of ACSL4 as a biomarker and potential therapeutic target in cancer, underscoring its significance in the context of (LUAD and the potential impact on the overall understanding of ferroptosis in cancer biology. Abnormal metabolic mechanisms associated with cancer ferroptosis offers the potential for developing latent effective therapeutic targets.

SLC7A11, a member of the solute carrier family 7, functions as an amino acid transporter, which can regulate ferroptosis [21]. Down-regulation of SLC7A11 inhibits GPX4 activity, causing an accumulation of lipid peroxides which triggers cell ferroptosis[[22,23]]. Recent researches have reported SLC7A11 is overexpressed in several solid malignancies, such as breast cancer, pancreatic cancer, ovarian cancer, and glioma, and closely related to the therapeutic drug resistance of cancers[[24–26]]. Therefore, due to its potential for developing novel cancer therapies, SLC7A11 has become a focal point in cancer treatment research.

This study aims to address a critical gap in the current understanding of LUAD progression by elucidating the regulatory function of ORC1 and its relationship with SLC7A11 in the context of ferroptosis inhibition. While previous research has implicated ORC1 dysregulation in various human cancers, including cervical cancer, breast cancer, and squamous cell carcinoma, its specific role in LUAD and its interaction with SLC7A11 remain relatively unexplored. The novelty of this study lies in its comprehensive investigation of the mechanistic links between ORC1, SLC7A11, and ferroptosis inhibition in LUAD pathogenesis, shedding light on potential therapeutic targets and diagnostic biomarkers for this aggressive malignancy. By explicitly defining the unique contribution of this study in addressing the underexplored aspects of LUAD biology, we aim to provide valuable insights that can serve as a foundation for developing more effective therapeutic strategies and diagnostic approaches for LUAD patients.

2. Results

2.1. ORC1 was up-regulated in LUAD patients and associated with poor prognosis of LUAD

To study the involvement of ORC1 in LUAD, the differential expression of ORC1 was tested between tumor and normal tissues in TCGA database. Results showed significant upregulation of ORC1 expression in LUAD patients (Fig. 1A), which was further confirmed in the GEO dataset (GSE19188, GSE31210) (Fig. 1B and C) and Western blot experiments in NSCLC cell lines (Fig. 1D).

Further, we performed ROC curve analysis and compared AUC values in TCGA database to evaluate the sensitivity as well as specificity of ORC1 in the diagnosis of LUAD. Additionally, we established a logistic regression model to further assess the effectiveness of ORC1 in LUAD diagnosis. At the end, through confusion matrix, the accuracy of classification result will be evaluated. The average values of accuracy were 0.953, 0.969, 0.987, 0.962 and 0.976 respectively(Fig. 1E). These results mean that ORC1 is effective in distinguishing LUAD samples from the normal control group. Subsequently, time-dependent ROC curve shows that the expression level

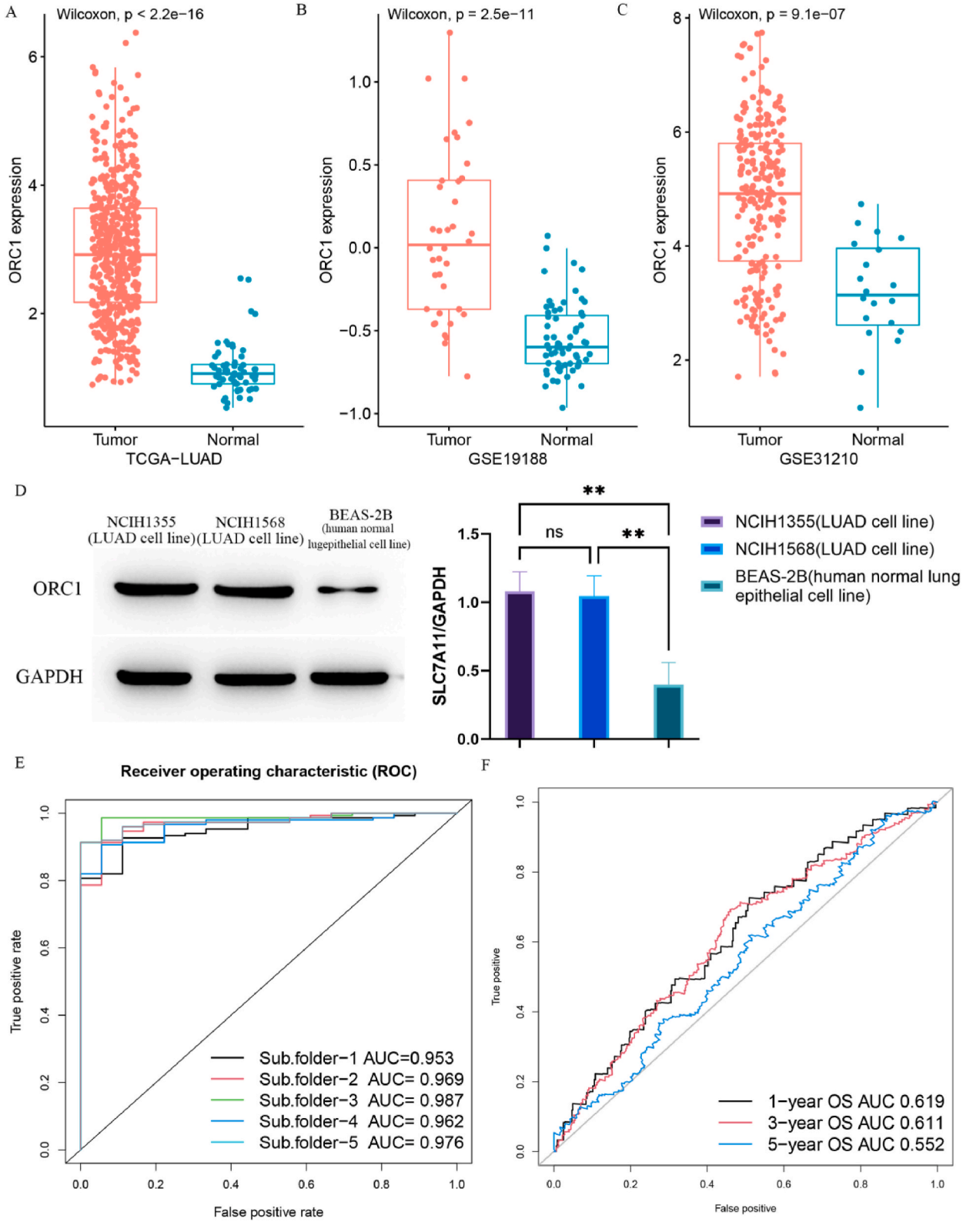

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Fig. 1. ORC1 expression is considerably higher in LUAD and strongly linked with a poor prognosis. (**A-C).** Box-whisker plots showing the expression levels of ORC1 in tumor tissues and normal tissues based on Wilcoxon test in TCGA and GEO database. **(D)** The protein expression levels of ORC1 in NCIH1355, NCIH1568 and BEAS-2B cell lines(Full, non-adjusted images were in supplementary material). **(E)** The ROC curve showing the AUC value of ORC1 in the five-fold cross-validation based on the data set of TCGA. **(F)** Time-dependent ROC curve showing the AUC value of ORC1 based on the data set of TCGA.

of ORC1 gene has certain prognostic value for the long-term survival of LUAD patients (Fig. 1F). For 1-year, 3-year, and 5-year survival, the area under the ROC curve is 0.619, 0.611, and 0.552, respectively. In brief, these outcomes strongly suggest the potential roles of ORC1 in the prognosis of patients with LUAD.

2.2. ORC1 knockdown inhibits the proliferation of LUAD cells in vitro by arresting the cell cycle

To demonstrate the function of ORC1 in LUAD, in vitro experiments were performed by inserting siRNA to knock down ORC1 in NCIH1355 and NCIH1568 cells. The CCK8 assay results showed that ORC1 knockdown significantly inhibited LUAD cell proliferation (Fig. 2A and B). We used RT-qPCR to examine the effect of sh-ORC1 on cell proliferation by assessing the changes of ki-67, a biomarker of cell proliferation. Results indicated that ORC1 knockdown resulted in the reduction of cell proliferation in NCIH1355 and NCIH1568 cells (Fig. 2C). These outcomes suggest that ORC1 knockdown can impair the in vitro growth of LUAD cell line.

2.3. SLC7A11 is the target gene of ORC1

To probe the mechanism of sh-ORC1 related cell death, NCIH1355 and NCIH1568 cells were treated with sh-ORC1 both in the absence and presence of various cell death-related inhibitors. Results showed that treatment with ferrostatin-1 (a potent inhibitor of ferroptosis) and deferoxamine (an iron-chelating agent) induced protective effects against sh-ORC1 induced growth inhibition, while treatment with chloroquine (a potent inhibitor of autophagy), necrostatin-1 (a potent inhibitor of necroptosis), or Z-VAD-FMK (a general caspase inhibitor) had no effect (Fig. 3A). These outcomes suggest that ferroptosis might be implicated in the mechanism of sh-ORC1 related cell death.

Gefitinib, a first-line treatment for LUAD patients, is associated with ferroptosis in LUAD. Results from CCK8 assays indicated that gefitinib treatment and sh-ORC2 can induced ferroptosis of LUAD cell lines and inhibited cell proliferation. However, when ORC1 was knocked down in combination with gefitinib treatment, LUAD cell proliferation inhibition and ferroptosis were further up-regulated compared to gefitinib treatment alone (Fig. 3B). These findings suggest that ORC1 knockdown may increase the sensitivity of LUAD cells to gefitinib-induced ferroptosis and cell death, highlighting the possibility of ORC1 as a potential target for LUAD treatment.

In previous studies, SLC7A11 was found to be associated with ferroptosis [22]. In this study, expression levels of SLC7A11 in LUAD related TCGA database and GEO dataset were analyzed and found to be significantly increased in LUAD patients ($R = 0.5$, $p = 1.05*10\hat{}-7$, Fig. 3C). Furthermore, Western blot results showed that protein levels of SLC7A11 in NSCLC cell lines were higher than those in normal human lung epithelial cell BEAS-2B cells (Fig. 3D). Survival analysis also revealed that SLC7A11 was not only differentially expressed but also related to LUAD survival (Fig. 3E). ORC1 overexpression plasmid was constructed, and NCIH1355 cells were transfected with it, leading to the induction of SLC7A11 expression (Fig. 3F). Conversely, siRNA-mediated ORC1 knockdown had the opposite effect, indicating that ORC1 can positively regulate the expression of SLC7A11. These results indicate that ORC1 have a significant function in modulating SLC7A11 expression in LUAD, possibly contributing to ferroptosis.

2.4. ORC1 promotes LUAD cell proliferation in a SLC7A11-dependent manner

Based on the observations made in the study, it appears that SLC7A11 may act as an effector of ORC1 in LUAD progression. To test this hypothesis, SLC7A11 was knocked down in NCIH1355 and NCIH1568 cells that were overexpressed with ORC1. Results showed that compared with NCIH1355 and NCIH1568 cells in the empty vector group, the cell proliferation rate in the ORC1 overexpression group was increased. However, this effect was abolished after SLC7A11 knockdown (ORC1+sh-SLC7A11) (Fig. 4A and B). Therefore, it

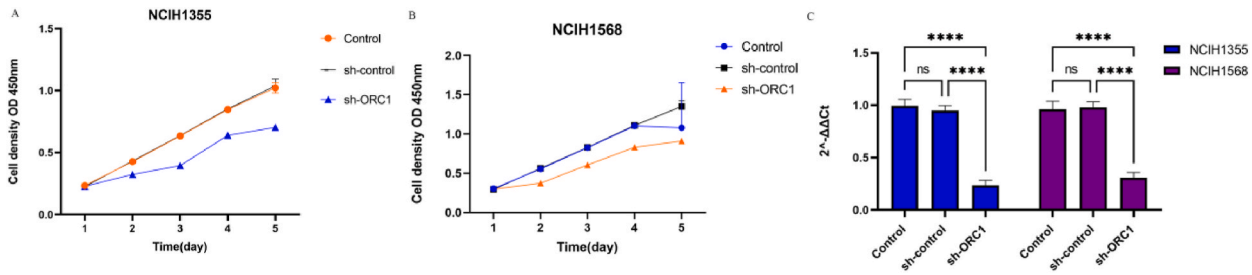

Fig. 2. Effects of ORC1 on LUAD cell lines proliferation and cell cycle. (A–B) The number of viable NCIH1355 and NCIH1568 cell density was analyzed using CCK8 assay. **(C)** In NCIH1355 and NCIH1568 cells, the results of RT-qPCR for ki-67 indicated ORC1 knockdown resulted in the reduction of cell proliferation.

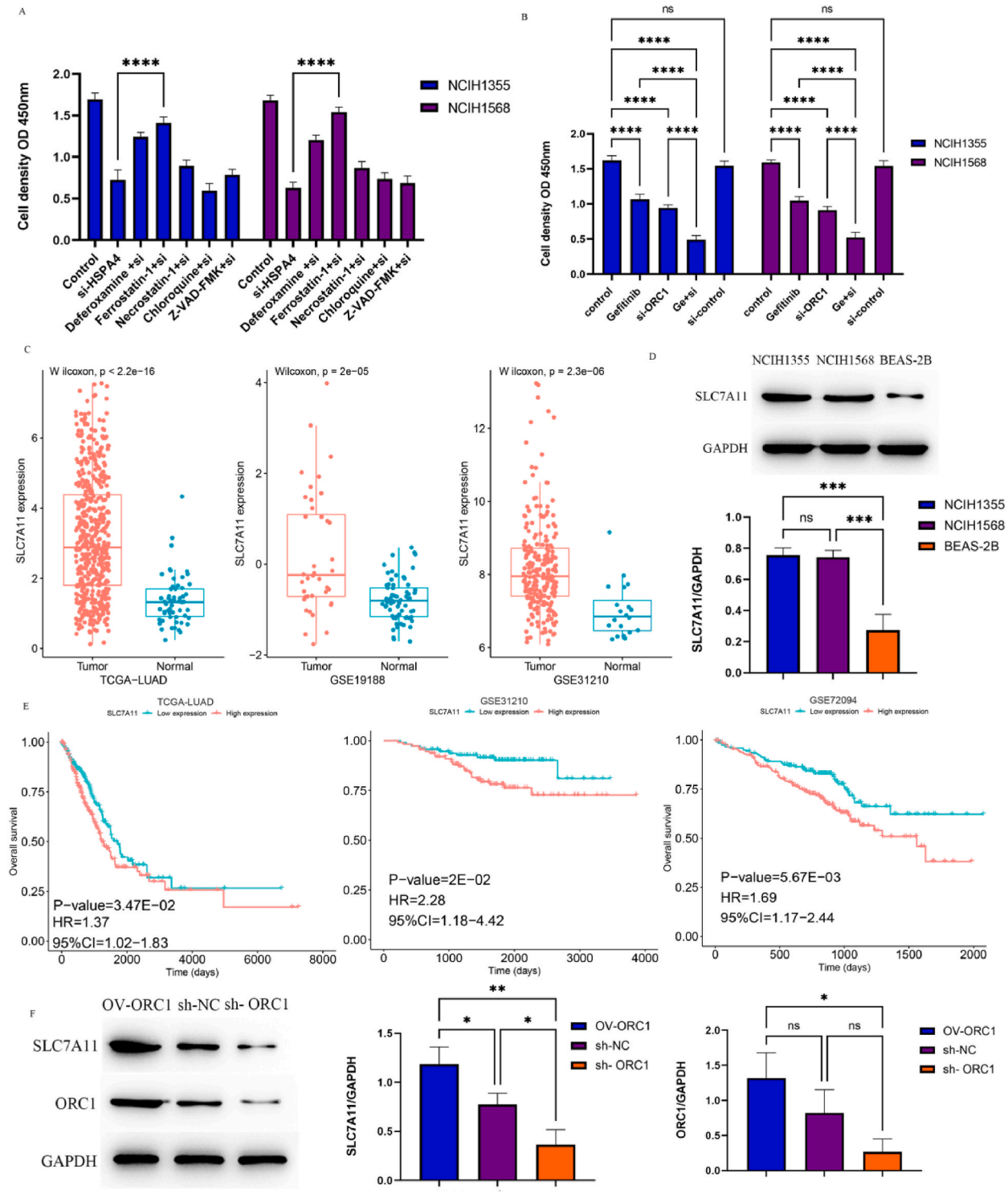

Fig. 3. SLC7A11 is the target gene of ORC1. (A) NCIH1355 and NCIH1568 cells were treated with OV-ORC1 in the absence as well as presence of a series of cell death related inhibitors. **(B)** The proliferation levels of NCIH1355 treated by sh-ORC1 or/and gefitinib. **(C)** The expression levels of SLC7A11 in LUAD and normal tissues in TCGA, GSE19188 and GSE31210 dataset. **(D)** The protein expression levels of SLC7A11 in NCIH1355, NCIH1568 and BEAS-2B cell lines(Full, non-adjusted images were in supplementary material). **(E)** Survival analysis of SLC7A11 expression in TCGA, GSE31210 and GSE72094 dataset. **(F)** The relationship of ORC1 and SLC7A11 were demonstrated by western-blot on the basis of sh-RNA(Full, non-adjusted images were in supplementary material).

can be concluded that knocking down ORC1 promotes gefitinib-induced LUAD cell death through an SLC7A11-dependent manner.

2.5. SLC7A11 knocking down promotes ferroptosis of LUAD induced by gefitinib

Results from CCK-8 assays showed that sh-SLC7A11 and gefitinib treatment can weaken the proliferative capacity of NCIH1355 cells compared to the control group, and sh-SLC7A11 combined with gefitinib treatment had a more significant inhibitory effect (Fig. 5A). Furthermore, RT-qPCR was utilized to measure the level of ACSL4 expression, a biomarker of ferroptosis, confirming that sh-SLC7A11 and gefitinib treatment could promote ferroptosis in NCIH1355 cells (Fig. 5B). Additionally, sh-SLC7A11 combined with gefitinib treatment had a more significant effect, indicating that they have a synergistic effect in inducing ferroptosis. Overall, the results suggest that targeting SLC7A11 in combination with gefitinib treatment could be an effective strategy to promote ferroptosis and suppress LUAD cell proliferation.

3. Discussion

LUAD is one of the deadliest and heterogeneous malignancies [1] with complex pathogenesis due to various genetic mutations [2], Nevertheless, clinically effective diagnostic and therapeutic methods for LUAD are still unavailable [6]. Hence, revealing potential mechanisms in LUAD pathogenesis will help to find novel biomarkers and targets for LUAD treatment. Prior researches have demonstrated upregulation of the protein ORC1 in many types of human cancers, contributing to cancer progression. For instance, the downregulation of ORC1 of glioma cell via shRNA impacted the expression of associated genes, leading to cell apoptosis promotion, cell cycle distribution alteration, proliferation suppression, and decreased migration and invasion. These effects were associated with the inhibition of the JNK and ERK signaling pathways [27]. The involvement of ORC1 as an oncogene in glioma is suggested. Moreover, the increased expression of ORC1 accelerates the progress of cervical cancer by promoting cell proliferation and reducing cell apoptosis [12]. However, the precise role of ORC1 in LUAD requires further investigation.

In this study, we evaluated the gene expression of ORC1 using the TCGA database, indicating the significant over-expression of ORC1 in LUAD patients, which was consistent with the trend in the GEO dataset. Subsequently, Western blot experiments were conducted to verify the above conclusions. Additionally, we discovered that ORC1 expression was significantly higher in advanced-stage cancer than in early-stage cancer, suggesting that ORC1 may have a potential function in the development and migration of tumors. In further establishing a logistic regression model, we found that using the ORC1 index for classifying LUAD samples resulted in an accuracy of 0.953. We concluded that ORC1 can serve as an effective biomarker for early screening of LUAD. Qian et al. previously reported that SLC7A11 as a novel prognostic biomarker for LUAD, which is in accordance with our results.[36].

To confirm the efficacy of ORC1 in LUAD, we knocked down the ORC1 and found that ORC1 knockdown significantly inhibit LUAD cell proliferation. To explore the mechanism of ORC1 leading to cell death, we treated NCIH1355 and NCIH1568 cells with sh-ORC1 inhibitors both in the presence and absence of cell-death related inhibitors The results suggested that sh-ORC1 can lead to ferroptosis in LUAD. Besides, sh-ORC1 can reinforce the curative effect of Gefitinib via activating ferroptosis.

Then, we constructed ORC1 overexpression plasmid and transfected it into NCIH1355 cells. We found that overexpression of ORC1 did induce the expression of SLC7A11, while siRNA-mediated ORC1 knockdown has the opposite effect, which indicates that ORC1 can positively regulate the expression of SLC7A11. These outcomes led us to believe that SLC7A11 an effector of ORC1 during LUAD progression. We tested this theory by knocking down SLC7A11 in NCIH1355 and NCIH1568 cells that were overexpressed with ORC1. Results showed that compared with NCIH1355 and NCIH1568 cells in the empty vector group, the cell proliferation rate in the ORC1 overexpression group was increased. However, this effect was abolished after SLC7A11 knockdown (ORC1+sh-SLC7A11) (Fig. 4A and B). Therefore, it can be concluded that knocking down ORC1 promotes gefitinib-induced LUAD cell death through an SLC7A11-dependent manner.

SLC7A11 belongs to the solute carrier family [25] and plays a vital role in promoting the synthesis of glutathione by mediating cystine uptake and glutamate release, which maintains cellular redox balance and protects against oxidative stress, thus preventing lipid peroxidation-induced cell death[[22,28]]. In malignancies such as breast cancer, ovarian cancer, liver cancer, lung cancer and other malignant tumors, SLC7A11 is greatly expressed, closely related to the growth, prognosis, metastasis and treatment. [[25,29,30]]. Conversely, Hu et al. [31] found that SLC7A11 inhibition offers potential therapeutic alternatives in KRAS-mutant LUAD cells,

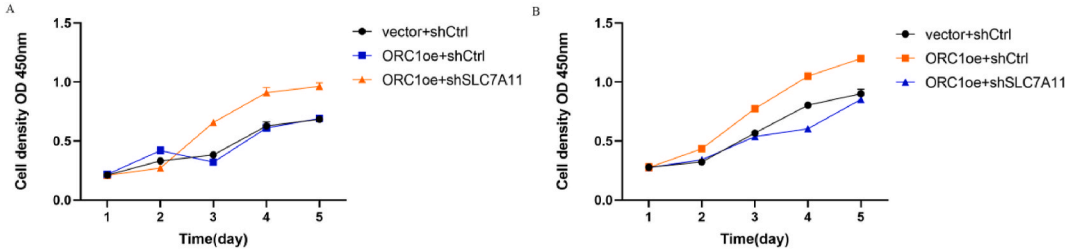

Fig. 4. ORC1 promotes LUAD cell line proliferation in a SLC7A11-dependent manner. (A–B). Compared with NCIH1355 and NCIH1568 cell line in the empty vector group, the cell proliferation rate in the ORC1 overexpression group was increased. However, this effect was abolished after SLC7A11 knockdown.

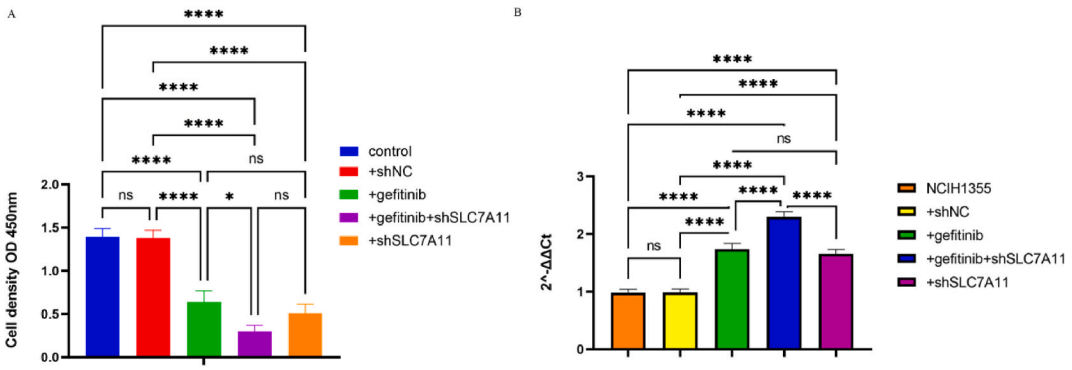

Fig. 5. SLC7A11 knocking down promotes ferroptosis of LUAD induced by gefitinib. (A) sh-SLC7A11 and gefitinib treatment can weaken the proliferative capacity of NCIH1355 cells compared to the control group, and sh-SLC7A11 combined with gefitinib treatment had a more significant inhibitory effect. **(B)** RT-qPCR for the level of ACSL4 expression, a biomarker of ferroptosis.

which is a currently incurable disease. Additionally, Xu et al. [32] identified an unexpected role of METTL3 in promoting LUAD tumor growth and inhibiting ferroptosis via stabilizing SLC7A11 m^6A modification. Pan et al. [33] found a crucial role of circP4HB in regulating ferroptosis through SLC7A11-mediated glutathione synthesis in LUAD. Therefore, this study added to previous studies by revealing a novel regulatory pathway of SLC7A11.

In the context of LUAD, the significance of SLC7A11 manifests in its multifaceted role in modulating redox balance, regulating ferroptosis, and impacting the cellular response to oxidative stress, which collectively contribute to cancer pathogenesis and therapeutic responsiveness. Our study unveils the intricate relationship between SLC7A11 and LUAD progression, particularly in the context of ferroptosis inhibition. The upregulation of SLC7A11 observed in LUAD patients and cell lines, coupled with its association with poor prognosis, underscores its potential as a crucial mediator of cancer cell survival and aggressiveness. Furthermore, our findings demonstrate that ORC1 positively regulates the expression of SLC7A11, highlighting the pivotal role of this regulatory axis in promoting LUAD proliferation and inhibiting ferroptosis. By shedding light on the direct involvement of SLC7A11 in LUAD biology and the intricate crosstalk with ORC1, our study accentuates the clinical relevance of targeting SLC7A11 as a potential therapeutic strategy for LUAD, opening avenues for more precise and effective treatment modalities in the context of ferroptosis regulation.

It is essential to consider the potential limitations of generalizing the findings of this study to the broader population of LUAD patients, particularly in light of the inherent heterogeneity of cancer. While the results obtained from our in vitro experiments and bioinformatics analyses provide valuable insights into the regulatory role of ORC1 and its interaction with SLC7A11 in promoting LUAD progression and inhibiting ferroptosis, extrapolating these findings to all LUAD patients should be approached with caution. The inherent heterogeneity of lung adenocarcinoma, encompassing diverse genetic mutations, tumor microenvironments, and patient-specific factors, may influence the applicability of these findings across the broader population of LUAD patients. Further studies encompassing a more comprehensive representation of LUAD patients, including diverse genetic subtypes and clinical characteristics, are warranted to enhance the generalizability and translational relevance of the observed associations.

However, this study has a few limitations. First, whether a direct interaction exists between ORC1 and SLC7A11 were not verified. Second, in vivo study was not involved in this study. One notable limitation pertains to the generalizability of the results, as the study predominantly focused on in vitro experiments and bioinformatics analyses. Extrapolating these findings to the broader population of LUAD patients should be performed with caution, given the inherent heterogeneity of cancer and the complex interplay of genetic, environmental, and clinical factors that can significantly influence disease progression. Moreover, potential confounding variables, such as variations in tumor microenvironments and patient-specific characteristics, could introduce biases that warrant consideration. Additionally, the study's exclusive focus on specific molecular pathways may overlook the potential influence of broader systemic and environmental factors on LUAD pathogenesis. As such, while the reported findings offer valuable mechanistic insights, the limitations underscore the need for comprehensive validation across diverse patient cohorts and consideration of potential confounders in future research endeavors. Additional confirmatory experiments may be needed to provide comprehensive validation of the findings. These experiments included alternative assays such as BrdU (5-bromo-2'-deoxyuridine) incorporation assays and cell counting with trypan blue exclusion to ensure robustness and reliability of the results. Despite these limitations, this study was the first to uncover the role of ORC1 in LUAD and its relationship with SLC7A11.

4. Conclusion

In summary, our findings indicate that ORC1 promotes LUAD cell proliferation and suppresses ferroptosis in a SLC7A11-dependent manner. These results imply that ORC1 could be a promising target or biomarker for LUAD diagnosis and treatment. Additionally, this study is s the first to reveal the role of ORC1 in LUAD as well as its relationship with SLC7A11 in LUAD.

5. Materials and methods

5.1. Data acquisition and collection

This study is designed as a translational research endeavor employing bioinformatics analyses, in vitro experiments utilizing human lung adenocarcinoma (LUAD) cell lines, and molecular biology techniques. The investigations aim to elucidate the regulatory role of ORC1 in the progression of LUAD and its interaction with SLC7A11, with a particular focus on ferroptosis inhibition. The transcriptome expression data for this study were download from TCGA database (LUAD: 483 tumors samples and 59 normal samples) [34], and GSE19188, GSE72094 and GSE31210 in the GEO database. For clinical information, overall survival (OS) was analyzed on the basis of Kaplan-Meier plots and Long rank test.

5.2. Cell culture

The human LUAD cell line NCIH1355, NCIH1568 and human normal lung epithelial cell line BEAS-2B were procured from the American Type Culture Collection and cultured in RPMI-164 (Solarbio, China) supplemented with 10 % fetal bovine serum (Gibco, Australia) and 100 unit/mL penicillin and streptomycin (Solarbio, China) at 37 °C in 5 % CO2 environment. All these cell lines were confirmed through short tandem repeat analysis. All experiments were carried out using mycoplasma free cells. Subsequently, the cells were subjected to specific treatments for the experimental procedures. For the LUAD cell lines, treatments included ORC1 knockdown using siRNA and overexpression of ORC1. Additionally, the cells were treated with sh-ORC1 both in the absence and presence of various cell death-related inhibitors, including ferrostatin-1 (a potent inhibitor of ferroptosis), deferoxamine (an iron-chelating agent), chloroquine (a potent inhibitor of autophagy), necrostatin-1 (a potent inhibitor of necroptosis), and Z-VAD-FMK (a general caspase inhibitor). Furthermore, the cells were treated with gefitinib, a first-line treatment for LUAD patients, to investigate its association with ferroptosis. For the normal lung epithelial cell line BEAS-2B, specific treatments and controls were applied to ensure consistency and comparability with the LUAD cell lines throughout the experimental procedures.

5.3. RT-qPCR analysis

Total RNA isolation was extract via Trizol reagent (Invitrogen, USA) and cDNAs synthesis was completed following the Moloney Murine Leukemia Virus Reverse Transcriptase kit (Madison, USA) according to the manufacturer's instructions. The quality and quantity of the isolated RNA were assessed using Nanodrop. RNA integrity and purity were evaluated by measuring the A260/A280 and A260/A230 ratios to ensure high-quality RNA samples for downstream applications. Quantification of ORC1, SLC7A11, and GAPDH was conducted on the basis of SYBR Premix Ex Taq kit (Takara Biotechnology) with an ABI-7500 Thermal Cycler (Applied Biosystems Inc., USA). All experiments were examined in triplicate. Primers for amplification included (forward, reverse): ATGGCACACTACCCCACAAG, CCGTTTACAGGCAGGGACTT (ORC1); TGGAACGAGGAGGTGGAGAA, TGGTGGACACAACAGGCTTT (SLC7A11); GCGACCGTGGAGATGGACAA, AGGTTGCCACTAAAGGGTTT (ki-67); and AATGGGCAGCCGTTAGGAAA, GCGCCCAATACGACCAAATC (GAPDH).

5.4. Western blot analysis

For western blotting, total protein was separated using a lysis buffer (Cell Signaling Technology, USA) which supplemented with phosphatase inhibitors (Roche, Switzerland). Additionally, protein lysates (35 mg protein) were subject to 8 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and then transferred onto a polyvinylidene fluoride (PVDF) membrane. Once transferred, the PVDF membranes were blocked with 5 % nonfat milk (BD Biosciences, 232,100, USA). Then the target protein was probed with the corresponding primary as well as secondary antibodies (1:200). In this research, primary antibodies used are: ORC1 (abcam, USA, ab251776, 1:1000), SLC7A11 (abcam, USA, ab307601, 1:1000), and GAPDH (Cell Signaling Technology, USA, 5174, 1:1000).

5.5. Cell proliferation assay

To determine the effect of ORC1 and SLC7A1 on proliferation, the CCK8 (Dojindo Laboratories, Japan) was performed in compliance with the manufacturer's instructions. In brief, cultured cell lines were inoculated in a 96-well plate at a concentration of 5 $\times 10^3$ cells/well. Following 24-h time intervals, 10 μl CCK8 solution was applied to each well. After additional 2.5 h of incubation, the plate was read on a microplate reader (SPECTROstar Nano, Germany).

5.6. Statistical analysis

Statistical analysis was performed with SPSS 23.0 (SPSS, USA) or GraphPad Prism 7 (GraphPad Prism, USA). Every results were confirmed in at least three independent experiments. Numerical data is reported as means ± standard deviation (SD) and significance of statistical analysis was conducted using two-tailed, unpaired Student's *t*-test or variance analysis. For the statistical analysis of experimental data involving more than two groups, one-way analysis of variance (ANOVA) was utilized to determine the significance of differences among the multiple experimental conditions. Bonferroni's post hoc analysis were applied for multiple comparisons to ascertain specific group differences where applicable. A P value < 0.05 was considered statistically significant.

Ethical statement

Not applicable.

Availability of data and materials

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

Consent for publication

Not Applicable.

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CRediT authorship contribution statement

Linlin Ming: Writing – review & editing, Writing – original draft, Resources, Conceptualization. **Zhendong Han:** Writing – review & editing, Writing – original draft, Software, Project administration. **Zhongwei Ai:** Writing – review & editing, Writing – original draft, Software, Data curation. **Xiaofeng Yang:** Writing – review & editing, Writing – original draft, Software, Data curation. **Fei Lin:** Writing – review & editing, Writing – original draft, Data curation. **Ning Zhang:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Wenbo Hao:** Writing – review & editing, Writing – original draft, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

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.

Acknowledgements

Not applicable.

Appendix A. Supplementary data

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