High SLC7A11 Expression Is Correlated With Poor Prognosis and Associated With Ferroptosis in Ovarian Cancer

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Research Article

Abstract

The current treatments of ovarian cancer (OC) do not yield satisfactory outcomes. Hence, it is necessary to find new treatment targets for OC. In this study, a comprehensive bioinformatic analysis was conducted to identify differentially expressed genes (DEGs) between OC and control tissues. Five datasets were downloaded from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) were screened by comparing gene expression between OC and control tissues. Module analysis of DEGs was performed on the STRING database and GEPIA. Kaplan Meier plotter and GEPIA database analysis the overall survival. Finally, SLC7A11 was found to be is the hubgene. And we confirm that the protein expression of SLC7A11 was increased in OC tissues. Analysis of a variety of tumor gene databases showed that SLC7A11 gene regulated the processes of OC. The low mutation rate of the gene (which were of amplified type) and high mRNA expression were associated with poor prognosis of OC patients.Using erastin-treated ovarian cancer (OC) cell lines, we examined the relationship between ferroptosis and OC. Results showed that OC tissues contained higher malondialdehyde (MDA) levels than normal tissues. Unlike normal ovarian epithelial cells which are not sensitive to erastin, the OC cell line, ES-2 is very sensitive to erastin. Here, we found that ferrostatin-1 treatment increased levels of reactive oxygen species (ROS), malondialdehyde, and SLC7A11 protein expression. These results provide an important theoretical basis for further studies into the role of SLC7A11, the effective biomarker and potential drug target, in the occurrence and development of OC.

Introduction

Ovarian cancer (OC) is one of the most lethal gynecological malignancy in China [1]. Given the lack of reliable biomarkers for early diagnosis and treatment of the disease, many patients with OC develop peritoneal metastases and have poor prognosis [2]. Platinum-based drugs are the most widely used chemotherapeutic agents to treat advanced OC. However, these drugs are associated with recurrent tumor resistance and side effects at high doses [3,4]. Improving the cure rate and long-term survival rate of OC patients in the high-risk group is key to improving the overall prognosis.

Several biochemical molecular markers related to tumor occurrence and development have been shown to be important for early tumor screening. However, many biomarkers are not tumor-specific [5]. Therefore, it is necessary to search for new specific diagnostic OC markers to improve early diagnosis of the disease. Currently, microarray technology and bioinformatic analysis have become important tools for exploring genetic or epigenetic variations during carcinogenesis and assessing cancer diagnosis and prognosis [6, 7]. Gene Expression Omnibus (GEO) is an international public repository that harbors microarrays, next-generation sequencing, and other forms of high-throughput functional genomic data [8, 9]. The publicly available data of cancer provide gene expression profiles and which can be utilized for early diagnosis, treatment, and prevention of various cancers[10, 11] .

In this study, we downloaded five OC chip datasets, GSE6008, GSE26712, GSE14001, GSE40595 and GSE105437 from the GEO database[12-16]. Differentially expressed genes (DEGs) were screened by

comparing gene expression between OC and control tissues. Module analysis of DEGs was performed using the STRING database and GEPIA.Then, we used Kaplan Meier plotter and GEPIA database analysis the overall survival to obtain the target genes which might become new biomarkers.Finally, we examined the association of ferroptosis with OC and provided an important experimental basis for the construction of erastin-resistant cell lines.

Materials & Methods

Microarray data

Five gene expression datasets (GSE6008, GSE26712,GSE14001,GSE40595 and GSE105437) were obtained from the NCBI Gene Expression Synthesis (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). The GSE6008 array data was submitted by Hendrix ND et al., and contains 99 OC tissues and 4 normal ovary tissues [12]. The GSE26712 array data was submitted by Bonome T et al., and includes 185 OC tissues and 10 normal ovary tissues [13]. Both datasets are based on the GPL96 platform. The GSE14001 array data was submitted by Tung CS et al., and comprised 20 OC tissues and 3 normal ovary tissues [14]. The GSE40595 array data was submitted by Yeung et al., and include 32 OC tissues and 6 normal ovary tissues [15]. The GSE105437 array data was submitted by Noh et al., including 10 OC tissues and 5 normal ovary tissues [16]. The GSE14001、GSE40595 and GSE105437 are based on the GPL570 platform.

Screening for DEGs

The Microarray datasets were using the R language software and annotation package. The ID corresponding to the probe name was converted into an international standard name for genes (gene symbol) and saved in a TXT file. Gene differential expression analysis was performed using the Limma package in the Bioconductor package (available online: http://www.bioconductor.org/). The related operating instruction codes were exported into R, and the DEGs between ovarian cancer and normal ovarian samples of the three microarray datasets were analyzed using the Limma software package. Samples with a corrected P-value of ⬚0.05 and log fold change (FC) ⬚0 were considered DEGs. The TXT results were preserved for subsequent analysis.

Survival analysis and RNA sequencing expression of core genes

Kaplan Meier-plotter is a commonly used website tool for assessing the effect of several genes on survival based on EGA, TCGA database and GEO (Affymetrix microarrays only). The log rank P value and hazard ratio (HR) with 95% confidence intervals were computed and plotted. To validate the DEGs, the GEPIA website was employed to analyze RNA sequencing expression data using thousands of samples from the GTEx projects and TCGA.

GEPIA database search

 When searching the GEPIA database, the "Boxplot" option was used to analyze the expression of *SLC7A11* mRNA in normal ovarian tissues and OC tissues. The "stage diagram" option was employed to

analyze the expression of *SLC7A11* mRNA at different TNM stages of OC tissues, whereas the "survival diagram" option was employed to analyze the association of *SLC7A11* with patient prognosis.

Specimen collection from patients

Tumor specimens were collected from OC patients undergoing primary surgical treatment who were not treated with chemotherapy at Beijing Obstetrics and Gynecology Hospital. A total of 3 tumors and 3 normal ovarian tissues were used to for immunohistochemistry、western blot analysis and malondialdehyde (MDA) assays.

Cell culture and reagents

Five OC cell lines (SKOV3, ES-2, A2780, COC1 and OVCAR3) and normal ovarian cell line (IOSE80) were obtained from the Procell Life science & Technology (Wuhan, China). These cell lines were cultured in high-glucose Dulbecco's modified Eagle's medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Sigma, USA), 100 units/ml penicillin and streptomycin (HyClone, USA). All cells were grown in a humidified incubator with 5% CO_2 at 37°C.

Immunohistochemistry (IHC)

Tissues were fixed with 4% paraformaldehyde (PFA), embedded in paraffin, and sliced into 5-μm thick sections, which were then deparaffinized with xylene and rehydrated in graded ethanol. For antigen retrieval, the sections were incubated in sodium citrate buffer (10 mM, pH 6.0) at 95°C for 20 min. After blocking with 5% goat serum in PBS, the sections were incubated overnight at 4°C with primary antibodies targeting the following proteins: SLC7A11/*SLC7A11* (D2M7A) Rabbit mAb (12691; Abcam; 1:50). For the subsequent steps, the sections were stained with SPlink Detection Kits (Biotin-Streptavidin HRP Detection Systems) (ZSGB-BIO), and the colors were developed using a DAB Kit (ZSGB-Bio) according to the manufacturer's instructions. The sections were briefly counterstained with hematoxylin, observed under a light microscope, and imaged with a digital camera.

Cell viability assay

OC cells were seeded in 96-well plate at a density of $1 \cdot 10^5$ cells/well in 100mL of culture medium. The cells were precultured for 36 hAt 36 h, 10mL of the Cell Counting Kit-8 (CCK8) (Solibor, Haimen, China) solution was added to each well and incubated for 1 h at 37°C and 5% CO_2 incubator. Subsequently, the absorbance of the solution was measured at a wavelength of 450nm using a SpectraMax M5 (Eppendorf, Hamburg, Germany).

ROS and MDA measurement

Intracellular ROS was evaluated by using 2,7'-dichlorodi-hydrofluorescein diacetate (H2DCFDA; Invitrogen). After the above-indicated treatments, cells were stained with the H2DCFDA for 10min, and

then washed twice with phosphate-buffered saline (PBS). Fluorescence of cells suspended in PBS was measured using a fluorescence spectrophotometer (Agilent, Santa Clara, CA, USA).

Cells were lysed and the supernatant (2mL) was mixed with 2mL of 0.6% thiobarbituric acid in a 10 mL tube. The tube was then kept in boiling water for 15min and placed on ice to cool down and the optical density was measured at 532nm. The results were expressed as nmol MDA/g protein.

Statistical analysis

The mRNA expression level of *SLC7A11* in OC and normal ovarian tissues was compared using the t test. One-way analysis of variance was used to compare differences in *SLC7A11* expression across different stages of OC. The Kaplan-Meier survival curve was employed to examine the relationship between SLC7A11 expression, variation and prognosis, and the survival rate between the two groups using Log-Rank test. The data obtained were analyzed using the online statistical analysis, and P<0.05 was considered statistically significant.

Results

Microarray data information and identification of DEGs

To identify DEGs, we performed background correction, and normalization of the OC expression microarray datasets GSE6008、GSE26712、GSE14001、GSE40595 and GSE105437. The differentially expressed genes in both sample datasets from the five microarrays is shown in Fig.1A. Then, we passed GEPIA verification and found that 36 molecules were up-regulated（Fig.1B）. In addition, Kaplan Meier plotter (http://kmplot.com/analysis) was utilized to identify survival data of 36 core genes. Results showed that 7 genes had a significantly worse survival rate while 29 had no effect on survival rate (*P*< 0.05, Fig.S1,Table 1).

High *SLC7A11* expression is correlated with poor prognosis

Analysis of the GEPIA database showed that the overall survival rate of the high *SLC7A11* mRNA expression group was significantly lower than those of the low *SLC7A11* mRNA expression group while 6 genes had no significant effect on survival rate（*P*< 0.05, Fig.2A）. This suggested that high *SLC7A11* mRNA expression may be linked to poor prognosis of OC patients. The expression of *SLC7A11* varied across different OC TNM stages, and it was slightly highly expressed in stage II, but not significantly (Fig.2B). Results of cBioPortal analysis indicated that among the 4 data sets from TCGA, the total mutation rate was 1%, all of which were amplified mutations. This showed that the mutation type of *SLC7A11* in OC was the amplification mutation, but the mutation rate was not high (Fig.2C,D). The STRING network-based protein interaction analysis was used to generate a PPI network to study the protein-protein interactions of *SLC7A11*. Results showed that upregulated *SLC7A11* was mainly involved in ferroptosis, protein digestion and absorption (*P*< 0.05, Fig.2F, Table 2).

OC is associated with ferroptosis

SLC7A11 gene encodes the SLC7A11 protein. Immunohistochemistry test revealed that SLC7A11 level was higher in ovarian tumors than in normal ovarian tissue (Fig.3A). Western blotting assay demonstrated that SLC7A11 expression in OC cells was significantly higher than in normal ovarian cells (Fig.3B). The degree of ferroptosis in cells was evaluated by testing levels of ROS and MDA. Malignant cells had higher levels of MDA than normal ovarian tissues (Fig.3C). Erastin is a classic ferroptosis activator in several cell types. To explore the sensitivity of different OC cell lines to erastin, we stimulated cells with different concentrations of erastin and determined their viability with the CCK8 assay. We found that ES-2, a widely used ovarian clear cell carcinoma cell line established in the late 1980s, was particularly sensitive to erastin. However，IOSE80 was relatively insensitive to erastin (Fig. 3D, E).

Biochemical changes in OC cells exposed to different treatments

To establish a model of ferroptosis in OC cell lines, we treated SKOV3, COC1, A2780 and OVCAR3 cells with different concentrations of erastin in the absence or presence of the small molecule Fer-1 and determined the viability of cell lines with CCK8 assay. Treatment of OC cells with erastin resulted in significant reduction of cell viability which was rescued by Fer-1 (Fig.4A, B). The levels of ROS and MDA in erastin+Fer-1 group were higher than those in the control group, but lower than those in the erastin group (Fig. 4C,4D). Results of SLC7A11 expression in different treated groups of OC cells are shown in Fig.4E.

Discussion

Although significant progress has been achieved in targeted therapy, immunotherapy and endocrine therapy, OC chemotherapy resistance is still the main reason for limiting patients' survival rates[3,4]. Hence, it is necessary to find new treatment targets for OC.

Five datasets were downloaded from the Gene Expression Omnibus (GEO) database. Differentially expressed genes (DEGs) were screened by comparing gene expression between OC and control tissues. Module analysis of DEGs was performed on the STRING database and GEPIA. Kaplan Meier plotter and GEPIA database analysis the overall survival. Finally, SLC7A11 was found that we need.Subsequently,we confirm that SLC7A11 protein was significantly higher in OC tissues than in normal ovarian tissues.

SLC7A11 is a cystine/glutamic acid xc-transport carrier which is responsible for the specific transport of cystine and glutamate [17]. It ingests extracellular cystine into the cell for the synthesis of glutathione (GSH), while transferring glutamic acid out of cells at a ratio of 1:1 [18]. It has been reported that SLC7A11-mediated cystine absorption and glutathione synthesis are closely related to tumor cell proliferation and growth [19,20]. SLC7A11 activity and expression were decreased due to GSH reduction, enhancement of ROS and lipid peroxidation [21].A critical modulator of intracellular redox balance is the system xc−transporter, which regulates the exchange of intracellular glutamate for extracellular cystine, an essential precursor for GSH synthesis. This complex consists of SLC7A11, a light-chain subunit that confers cystine transport function [22]. This is attributed to cis-acting transcriptional regulatory elements present in the SLC7A11 promoter, which contains an antioxidant response element (ARE) principally

recognized by NRF2 [23], and the amino acid response element (AARE), which is bound by ATF4 [24], a major player in the integrated stress response and oxidative stress response. Expression of SLC7A11 is deregulated in multiple cancers. Indeed, overexpression of SLC7A11 has been reported in nonsmall cell lung cancer, gastric cancer, and pancreatic Cancer and is associated with poor outcomes [23-25]. However, its role in ovarian cancer has not been reported.

We found that the high expression of SLC7A11 was linked to poor overall survival of patients, but not significantly related to tumor staging, indicating that SLC7A11 can be a potential molecular marker for early diagnosis and prognosis prediction of OC. Further analysis of tissue RNA sequencing results revealed that the mutation rate of the SLC7A11 gene in OC tissue was low and the type of mutation was mainly amplification. This mutation was not significantly correlated with the patient's prognosis.

Numerous basic and clinical studies have found that the level of lipid reactive oxygen species in tumor cells is higher than that of normal cells [26,27].Recently, ferroptosis, which involves lipid reactive oxygen species, has been proposed to be a potential therapeutic target for cancer[28].

Here, we found that ferroptosis was associated with ovarian cancer and ES-2 was very sensitive to erastin. Our study provides an experimental basis for the construction of erastin-resistant cell lines. The results indicate that ferroptosis-inducing agents are potential therapeutic agents in OC. After treatment of OC cells with erastin, ROS levels increased, which upregulated MDA level, an indicator of lipid peroxidation that enhances SLC7A11 expression. Fer-1 inhibits ferroptosis by preventing lipid peroxidation [29]. Fer-1 alleviated the increase in ROS, MDA, and decrease of SLC7A11 induced by erastin. This suggests that ROS, malondialdehyde, and SLC7A11 play important roles in OC, as well as potential therapeutic targets for future research.

In summary, our study identified SLC7A11 involved in ferroptosis in OC. According to the functional analysis and survival analysis, SLC7A11 may serve as potential biomarkers or drug targets contributing to improve the survival rates of OC patients. In the next plan, we will continue to explore the underlying mechanisms and new drugs of SLC7A11 in more depth.

Declarations

Conflicts of interest: The authors declare no conflict of interest.

Ethics approval:The study was approved by the Ethics Committee of Beijing obstetrics and Gynecology Hospital Affiliated to Capital Medical University.

Consent to participate: Not applicable.

Consent for publication: All authors consent to the publication of this study.

Availability of data and material: All data is available under reasonable request.

Code availability: Not applicable.

The trial registration number (TRN) and date of registration: Our study is not a clinical trial ,so the registration number and date of registration are not required.

Authors' contributions: Jinwei Miao designed and guided this research. Chunyu Xu、Xinyu Liu and Yubo Zhang contributed to collection of data. Yanqin Zhang, Xiangyu Chang and Di Wu performed the statistical analysis. Mengqi Deng performed data analysis and wrote the first draft of the manuscript. Jinwei Miao commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with Ethical Standards

1. Disclosure of potential conflicts of interest: The authors declare no conflict of interest.
2. Research involving Human Participants and/or Animals: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.
3. Informed consent: Informed written consent was obtained from each subject or each subject's guardian.

References

[1]Li A, Xie R, Zhi Q, Deng Y, Wu Y, Li W, Yang L, Jiao Z, Luo J, Zi Y, Sun G, Zhang J, Shi Y, Liu J (2018) BRCA germline mutations in an unselected nationwide cohort of Chinese patients with ovarian cancer and healthy controls. Gynecol Oncol 151(1):145-152. https://doi.10.1016/j.ygyno.2018.07.024. Epub 2018 Aug 2.

[2]Feng H, Gu ZY, Li Q, Liu QH, Yang XY, Zhang JJ (2019) Identification of significant genes with poor prognosis in ovarian cancer via bioinformatical analysis. J Ovarian Res 12(1):35.https://doi.10.1186/s13048-019-0508-2.

[3]Pignata S, Scambia G, Bologna A, Signoriello S, Vergote IB, Wagner U, Lorusso D, Murgia V, Sorio R, Ferrandina G, Sacco C, Cormio G, Breda E, Cinieri S, Natale D, Mangili G, Pisano C, Cecere SC, Di Napoli M, Salutari V, Raspagliesi F, Arenare L, Bergamini A, Bryce J, Daniele G, Piccirillo MC, Gallo C, Perrone F (2017) Randomized controlled trial testing the efficacy of platinum-free interval prolongation in advanced

ovarian cancer: The MITO-8, MaNGO, BGOG-Ov1, AGO-Ovar2.16, ENGOT-Ov1, GCIG Study. J Clin Oncol 35(29):3347-3353. https://doi.10.1200/JCO.2017.73.4293. Epub 2017 Aug 21.

[4]Harris FR, Zhang P, Yang L, Hou X, Leventakos K, Weroha SJ, Vasmatzis G, Kovtun IV (2019) Targeting HER2 in patient-derived xenograft ovarian cancer models sensitizes tumors to chemotherapy. Mol Oncol 13(2):132-152. https://doi.10.1002/1878-0261.12414. Epub 2018 Dec 21.

[5]European Colorectal Cancer Screening Guidelines Working Group, von Karsa, L., Patnick, J., Segnan, N., Atkin, W., Halloran, S., Lansdorp-Vogelaar, I., Malila, N., Minozzi, S., Moss, S., Quirke, P., Steele, R. J., Vieth, M., Aabakken, L., Altenhofen, L., Ancelle-Park, R., Antoljak, N., Anttila, A., Armaroli, P., Arrossi, S., … Valori, R. (2013) European guidelines for quality assurance in colorectal cancer screening and diagnosis: overview and introduction to the full supplement publication. Endoscopy 45(1):51–59. https://doi.org/10.1055/s-0032-1325997

[6]Peterson, J. F., Aggarwal, N., Smith, C. A., Gollin, S. M., Surti, U., Rajkovic, A., Swerdlow, S. H., & Yatsenko, S. A. (2015) Integration of microarray analysis into the clinical diagnosis of hematological malignancies: How much can we improve cytogenetic testing?. Oncotarget 6(22):18845–18862. https://doi.org/10.18632/oncotarget.4586

[7]Cooper C. S. (2001) Applications of microarray technology in breast cancer research. Breast cancer research 3(3):158–175. https://doi.org/10.1186/bcr291

[8]Ahlfors, H., Anyanwu, N., Pakanavicius, E., Dinischiotu, N., Lana-Elola, E., Watson-Scales, S., Tosh, J., Wiseman, F., Briscoe, J., Page, K., Fisher, E., & Tybulewicz, V. (2019) Gene expression dysregulation domains are not a specific feature of Down syndrome. Nature communications 10(1):2489. https://doi.org/10.1038/s41467-019-10129-9

[9]Wang, Z., Monteiro, C. D., Jagodnik, K. M., Fernandez, N. F., Gundersen, G. W., Rouillard, A. D., Jenkins, S. L., Feldmann, A. S., Hu, K. S., McDermott, M. G., Duan, Q., Clark, N. R., Jones, M. R., Kou, Y., Goff, T., Woodland, H., Amaral, F., Szeto, G. L., Fuchs, O., Schüssler-Fiorenza Rose, S. M., … Ma'ayan, A. (2016) Extraction and analysis of signatures from the Gene Expression Omnibus by the crowd. Nature communications 7:12846. https://doi.org/10.1038/ncomms12846

[10]Forés-Martos, J., Catalá-López, F., Sánchez-Valle, J., Ibáñez, K., Tejero, H., Palma-Gudiel, H., Climent, J., Pancaldi, V., Fañanás, L., Arango, C., Parellada, M., Baudot, A., Vogt, D., Rubenstein, J. L., Valencia, A., & Tabarés-Seisdedos, R. (2019) Transcriptomic metaanalyses of autistic brains reveals shared gene expression and biological pathway abnormalities with cancer. Molecular autism 10:17. https://doi.org/10.1186/s13229-019-0262-8

[11]Xu, Z., Zhang, Q., Luh, F., Jin, B., & Liu, X. (2019) Overexpression of the ASPM gene is associated with aggressiveness and poor outcome in bladder cancer. Oncology letters 17(2):1865–1876. https://doi.org/10.3892/ol.2018.9762

[12]Bonome, T., Levine, D. A., Shih, J., Randonovich, M., Pise-Masison, C. A., Bogomolniy, F., Ozbun, L., Brady, J., Barrett, J. C., Boyd, J., & Birrer, M. J. (2008) A gene signature predicting for survival in suboptimally debulked patients with ovarian cancer. Cancer research 68(13):5478–5486. https://doi.org/10.1158/0008-5472.CAN-07-6595

[13]Tung, C. S., Mok, S. C., Tsang, Y. T., Zu, Z., Song, H., Liu, J., Deavers, M. T., Malpica, A., Wolf, J. K., Lu, K. H., Gershenson, D. M., & Wong, K. K. (2009) PAX2 expression in low malignant potential ovarian tumors and low-grade ovarian serous carcinomas. Modern pathology : an official journal of the United States and Canadian Academy of Pathology 22(9):1243–1250. https://doi.org/10.1038/modpathol.2009.92

[14]Yeung, T. L., Leung, C. S., Wong, K. K., Samimi, G., Thompson, M. S., Liu, J., Zaid, T. M., Ghosh, S., Birrer, M. J., & Mok, S. C. (2013) TGF-β modulates ovarian cancer invasion by upregulating CAF-derived versican in the tumor microenvironment. Cancer research 73(16): 5016–5028. https://doi.org/10.1158/0008-5472.CAN-13-0023

[15]Hendrix, N. D., Wu, R., Kuick, R., Schwartz, D. R., Fearon, E. R., & Cho, K. R. (2006) Fibroblast growth factor 9 has oncogenic activity and is a downstream target of Wnt signaling in ovarian endometrioid adenocarcinomas. Cancer research 66(3):1354–1362. https://doi.org/10.1158/0008-5472.CAN-05-3694

[16]Noh, K., Mangala, L. S., Han, H. D., Zhang, N., Pradeep, S., Wu, S. Y., Ma, S., Mora, E., Rupaimoole, R., Jiang, D., Wen, Y., Shahzad, M., Lyons, Y., Cho, M., Hu, W., Nagaraja, A. S., Haemmerle, M., Mak, C., Chen, X., Gharpure, K. M., … Sood, A. K. (2017) Differential Effects of EGFL6 on Tumor versus Wound Angiogenesis. Cell reports 21(10):2785–2795. https://doi.org/10.1016/j.celrep.2017.11.020

[17]Doll, S., Freitas, F. P., Shah, R., Aldrovandi, M., da Silva, M. C., Ingold, I., Goya Grocin, A., Xavier da Silva, T. N., Panzilius, E., Scheel, C. H., Mourão, A., Buday, K., Sato, M., Wanninger, J., Vignane, T., Mohana, V., Rehberg, M., Flatley, A., Schepers, A., Kurz, A., … Conrad, M. (2019) FSP1 is a glutathione-independent ferroptosis suppressor. Nature 575(7784):693–698. https://doi.org/10.1038/s41586-019-1707-0

[18]Sharma, M., & Anirudh, C. R. (2019) In silico characterization of residues essential for substrate binding of human cystine transporter, xCT. Journal of molecular modeling 25(11):336. https://doi.org/10.1007/s00894-019-4233-y

[19]Lang, X., Green, M. D., Wang, W., Yu, J., Choi, J. E., Jiang, L., Liao, P., Zhou, J., Zhang, Q., Dow, A., Saripalli, A. L., Kryczek, I., Wei, S., Szeliga, W., Vatan, L., Stone, E. M., Georgiou, G., Cieslik, M., Wahl, D. R., Morgan, M. A., … Zou, W. (2019) Radiotherapy and Immunotherapy Promote Tumoral Lipid Oxidation and Ferroptosis via Synergistic Repression of SLC7A11. Cancer discovery 9(12):1673–1685. https://doi.org/10.1158/2159-8290.CD-19-0338

[20]Badgley, M. A., Kremer, D. M., Maurer, H. C., DelGiorno, K. E., Lee, H. J., Purohit, V., Sagalovskiy, I. R., Ma, A., Kapilian, J., Firl, C., Decker, A. R., Sastra, S. A., Palermo, C. F., Andrade, L. R., Sajjakulnukit, P., Zhang, L., Tolstyka, Z. P., Hirschhorn, T., Lamb, C., Liu, T., … Olive, K. P. (2020) Cysteine depletion induces pancreatic

tumor ferroptosis in mice. Science (New York, N.Y.) 368(6486):85–89.
https://doi.org/10.1126/science.aaw9872

[21]Jiang, L., Kon, N., Li, T., Wang, S. J., Su, T., Hibshoosh, H., Baer, R., & Gu, W. (2015) Ferroptosis as a p53-mediated activity during tumour suppression. Nature 520(7545):57–62.
https://doi.org/10.1038/nature14344

[22]Martin, L., & Gardner, L. B. (2015) Stress-induced inhibition of nonsense-mediated RNA decay regulates intracellular cystine transport and intracellular glutathione through regulation of the cystine/glutamate exchanger SLC7A11. Oncogene 34(32):4211–4218.
https://doi.org/10.1038/onc.2014.352

[23]Yang, Q., Li, K., Huang, X., Zhao, C., Mei, Y., Li, X., Jiao, L., & Yang, H. (2020) lncRNA SLC7A11-AS1 Promotes Chemoresistance by Blocking SCFβ-TRCP-Mediated Degradation of NRF2 in Pancreatic Cancer. Molecular therapy. Nucleic acids 19:974–985. https://doi.org/10.1016/j.omtn.2019.11.035

[24]Wang, S. F., Chen, M. S., Chou, Y. C., Ueng, Y. F., Yin, P. H., Yeh, T. S., & Lee, H. C. (2016) Mitochondrial dysfunction enhances cisplatin resistance in human gastric cancer cells via the ROS-activated GCN2-eIF2α-ATF4-xCT pathway. Oncotarget 7(45):74132–74151. https://doi.org/10.18632/oncotarget.12356

[25]Liu, Y., Fan, X., Zhao, Z., & Shan, X. (2020) LncRNA SLC7A11-AS1 Contributes to Lung Cancer Progression Through Facilitating TRAIP Expression by Inhibiting miR-4775. OncoTargets and therapy 13:6295–6302. https://doi.org/10.2147/OTT.S253082

[26]Li, F., Zhao, X., Sun, R., Ou, J., Huang, J., Yang, N., Xu, T., Li, J., He, X., Li, C., Yang, M., & Zhang, Q. (2020) EGFR-rich extracellular vesicles derived from highly metastatic nasopharyngeal carcinoma cells accelerate tumour metastasis through PI3K/AKT pathway-suppressed ROS. Journal of extracellular vesicles 10(1):e12003. https://doi.org/10.1002/jev2.12003

[27]Oddone, N., Boury, F., Garcion, E., Grabrucker, A. M., Martinez, M. C., Da Ros, F., Janaszewska, A., Forni, F., Vandelli, M. A., Tosi, G., Ruozi, B., & Duskey, J. T. (2020) Synthesis, Characterization, and In Vitro Studies of an Reactive Oxygen Species (ROS)-Responsive Methoxy Polyethylene Glycol-Thioketal-Melphalan Prodrug for Glioblastoma Treatment. Frontiers in pharmacology 11:574.
https://doi.org/10.3389/fphar.2020.00574

[28]Wu, Y., Yu, C., Luo, M., Cen, C., Qiu, J., Zhang, S., & Hu, K. (2020) Ferroptosis in Cancer Treatment: Another Way to Rome. Frontiers in oncology 10:571127. https://doi.org/10.3389/fonc.2020.571127

[29]Liu, P., Feng, Y., Li, H., Chen, X., Wang, G., Xu, S., Li, Y., & Zhao, L. (2020) Ferrostatin-1 alleviates lipopolysaccharide-induced acute lung injury via inhibiting ferroptosis. Cellular & molecular biology letters 25:10. https://doi.org/10.1186/s11658-020-00205-0
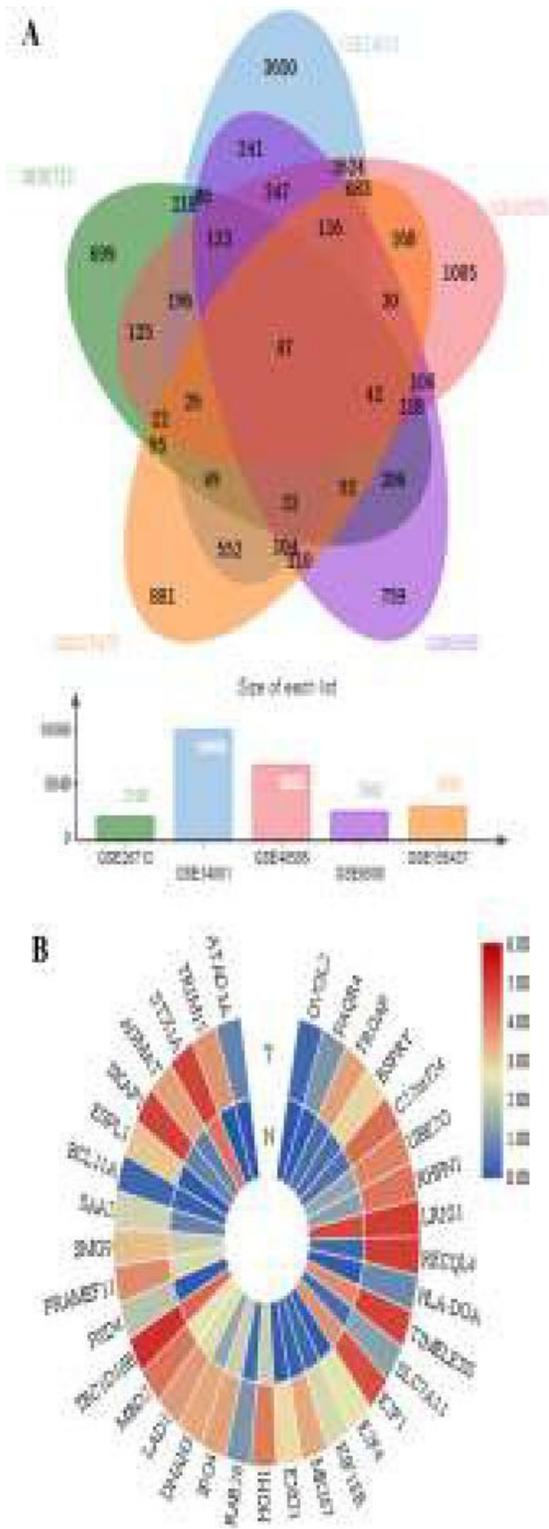
Figures

Figure 1

Microarray data information and identification of DEGs. A.Venn diagram of up-regulated genes in ovarian cancer.B.Heat map representation of up-regulated genes expression profile OC tumor samples and paired normal tissues determined by GEPIA.
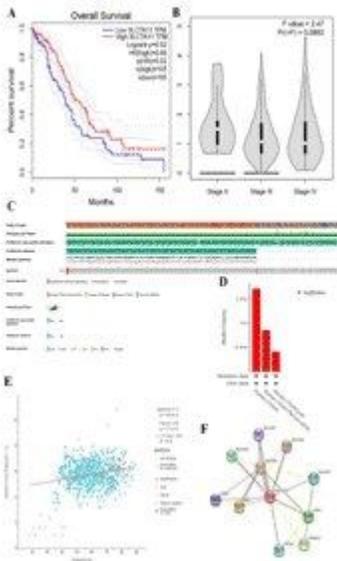
Figure 2

High SLC7A11 expression is correlated with poor prognosis.A.Survival curves comparing the high and low expression of SLC7A11 in OC by the GEPIA database(n=210)(P=0.02). B.The expression of SLC7A11 varied across different OC TNM stages by the GEPIA database(n=210)(P=0.0862). C and D.cBioPortal analysis the 4 different OC data sets from TCGA. F.The SLC7A11 protein function partner network was indicated by String analysis.
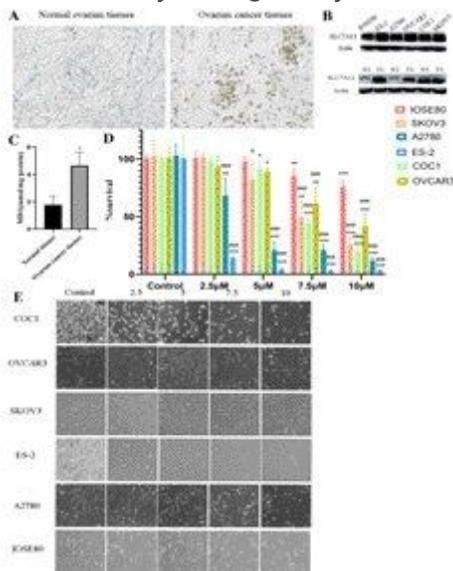

Figure 3

OC is associated with ferroptosis. A. Immunohistochemistry test was performed to assess the expression levels of SLC7A11 protein. B. Western blot assay was performed to assess the expression levels of SLC7A11 in OC cells as well as normal ovarian cells and ovarian tumors as well as normal ovarian tissue. All the data were repeated at least three times. C. The degree of ferroptosis was evaluated by testing the levels of malondialdehyde (MDA).Data are presented as mean± SD (n=3). D. Microscopic images in different (0,2.5,5,7.5,10 μ M) treated groups for 36h (100×). E. CCK8 assay in different (0,2.5,5,7.5,10 μ M)

treated groups over time. The results were compared with the control group and represented as the percentage of the control value. Data are presented as mean± SD (n=4). Compared with the control group, *p<0.05,**p<0.01, **p<0.001,and compared with IOSE80 cell,#p<0.05,##p<0.01, ###p<0.001
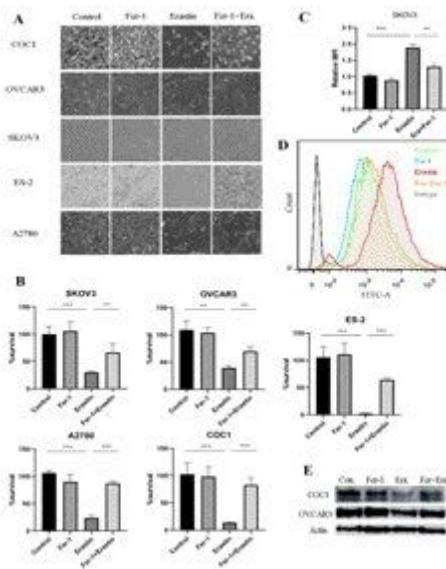

Figure 4

SLC7A11 overexpression inhibits ferroptosis of OC cells.A.Microscopic images in different treated groups for 36h (100×).B.CCK8 assay in different treated groups over time. The results were compared with the control group and represented as the percentage of the control value. C and D.Lipid ROS in erastin-treated SKOV3 cells were measured by Total Reactive Oxygen Species (ROS) Assay Kit 520 nm staining coupled to flow cytometry. The representative images (C) and the statistical results (D)were shown.E.Western blot assay was performed to assess the expression levels of SLC7A11 after the COC1 as well as OVCAR3 cells were treated for 48h. Data are presented as mean± SD (n=4). Compared with the control group, *p<0.05,**p<0.01, **p<0.001

Supplementary Files

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- FigS12.eps