Acyl-CoA Medium-Chain Synthetase-3 Promotes Chemosensitivity of Ovarian Cancer through the Inhibition of PI3K/AKT Signaling Pathway

Yuanyuan An

Capital Medical University

Hua Duan (✉ duanhua@ccmu.edu.cn)

Capital Medical University

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Abstract

Introduction: Dysregulation of fatty acid metabolism often occurs in tumor, which mainly constitutes of fatty acid synthesis and oxidation. In recent years, studies found that fatty acid metabolism participated in regulation of tumor immune microenvironment, which further influenced the progress of cancer. Thus, it is important to explore the key fatty acid metabolism-related molecules, which not only affects the prognosis of ovarian cancer, but also shows a close correlation with immune microenvironment of cancer.

Methods: Database from TCGA was used to explore the fatty acid metabolism-related molecules, which correlated with the prognosis of ovarian cancer using univariate and multivariate cox proportional regression model. Nomogram was constructed to predict the prognostic probability based on ACSM3 and clinicopathological parameters. GDSC database was used to investigate the chemosensitivity of ovarian cancer cells. The correlation between ACSM3 and immune status of ovarian cancer was analyzed by TIMER and TISIDB online tools. In addition, CCK8 assay was used to investigate the chemosensitivity of ovarian cancer cells, real time-PCR and western blot were used to investigate the expression of chemoresistance-related genes.

Results: ACSM3 worked as an independent favorable prognostic molecule through univariate and multivariate cox regression analysis. For the use in clinical, nomogram was constructed, and higher expression of ACSM3 showed better prognosis. We found that ACSM3 could regulate PI3K/AKT signaling, and GDSC database showed that PI3K/AKT inhibitor could promote the chemosensitivity of ovarian cancer cells. In addition, the expression of ACSM3 showed significantly correlated with the immune status of ovarian cancer. In vitro experiments showed that ACSM3 can promote the chemosensitivity of ovarian cancer cells by inhibiting PI3K/AKT signaling pathway.

Conclusion: Our results showed that ACSM3 acted as a favorable prognostic-related biomarker for ovarian cancer, which could promote chemosensitivity of ovarian cancer through inhibiting PI3K/AKT signaling pathway. This might be due to participate in regulating immune status of ovarian cancer microenvironment.

1. Introduction

As one of the most common malignant tumors in women, ovarian cancer ranking the third incidence rate places after cervical cancer and endometrial cancer. According to the statistics, although ovarian cancer didn't account for the top ten new female cancer cases in 2020, the mortality rate ranked the fifth, up to 5%, and the 5-year survival rate is only 44%(1). Due to lack of symptoms in early stage, makes the diagnosis for ovarian cancer is very difficult and most of the patients are in advanced stage. Fatty acid metabolism recombination is very important in cancer microenvironment, which has been suggested that fatty acid metabolism-related genes might work as a prognostic factor for ovarian cancer (2-4).

Dysregulation of fatty acid metabolism often occurs in cancer, which may influence the prognosis and biological behaviors of cancer, such as chemosensitivity and metastasis. FABP5 induced the lymph node metastasis through regulation of fatty acid synthesis, which influenced the prognosis of cervical cancer(5). Studies showed that fatty acid metabolism participated in the progression of ovarian cancer. SIK2 induced fatty acid synthesis through SREBP1c and thus increased transcription of FASN, which further promoted the progression of ovarian cancer(6). As we all known, acetyl-CoA is the important material for de novo synthesis of fatty acid, which comes from citric acid derived from tricarboxylic acid cycle, or acetic acid from outside with catalyzing by acetyl CoA synthetase(7). ACSM3 is a member of acyl-CoA medium chain synthetase family, which significantly correlated with fatty acid metabolism. It is found that ACSM3 could work as a biomarker and therapeutic target for hepatocellular carcinoma and malignant melanoma(8, 9). However, whether ACSM3 could work as the prognostic biomarker for ovarian cancer has not been reported yet.

In recent years, dysregulation of fatty acid metabolism correlated with the progression of cancer, which might due to its close correlation with the regulation of tumor immune microenvironment. RIPK3 was down-regulated in hepatocellular carcinoma-associated macrophages, thus enhanced the polarization of M2 macrophages. The deficiency of RIPK3 in TAMs inhibited caspase1 mediated cleavage of PPAR, thus activated PPAR and promoted fatty acid metabolism(10). Studies have found that FASN was closely related to a high degree of immunosuppression in the microenvironment of ovarian cancer. High expression of FASN in ovarian cancer ascites co-cultured with DCs and T cells could inhibit immune ability. This was through increasing high concentration of lipid, inhibiting tumor infiltrating DCs, supporting T cells dysfunction and inhibiting T cell function(11). In addition, study showed that ACSM3 was potentially associated with immune exclusion in malignant melanoma(8). However, there is no study about ACSM3 in regulating immune microenvironment of ovarian cancer. In this study, we found ACSM3 could act as the novel prognostic-related biomarkers for ovarian cancer, which significantly correlated with immune status, including immune cells and immune checkpoint inhibitors.

2. Methods

2.1 Data download and processing

The metabolism-related genes were collected from the Molecular Signature Database, which 396 genes were viewed as the candidate genes, including gene set M6999 (fatty acid beta oxidation), M10250 (fatty acid catabolic process), M11873 (fatty acid beta oxidation using acyl coa dehydrogenase), M12385 (fatty acid beta oxidation using acyl coa oxidase), M13591 (fatty acid biosynthetic process), M14568 (fatty acid oxidation) and M17829 (fatty acid metabolic process)(12). The expression of ACSM3 in ovary tissues, ovarian cancer tissues and borderline ovarian cancer tissues, and the relationship between ACSM3 and clinicopathological parameters were analyzed by Oncomine database. The analysis of ACSM3 expression in pan-cancer was downloaded from GEPIA. Firehose was used to collect the normalized gene expression and clinical information of ovarian cancer from TCGA dataset. The two independent datasets for ovarian cancer with accession number GSE30161 and GSE63885 were

downloaded from GEO datasets used for the validation, based on HGU133 Plus 2 platform(13, 14). In order to normalize the raw dataset, we used the Robust Multi-array Average method(15).

2.2 Development and construction of nomogram

The nomogram was constructed by R software (version 4.1.0) with the “rms” package. The C-index (concordance index) of the calibration plot was calculated to demonstrate the manifestation of nomogram. Both C-index and calibration plot represented the estimation of prognostic accuracy value for nomogram.

2.3 Investigate the DEGs in different expression of ACSM3

The differential expression genes were analyzed by R studio using “limma” packages, and 82 genes were identified (Table S2). The adjust p-value < 0.05 and absolute value of fold changes > 1.5 were set as the cut-off criteria. PPI network was constructed by String online database, and GO and KEGG analysis were analyzed by DAVAD online database.

2.4 Chemotherapeutic response

To analyze the inhibitor of PI3K/AKT signaling pathway, namely Apitolisib, in regulating the chemosensitivity of ovarian cancer using pharmacogenomics database of Genomics of Drug Sensitivity in Cancer (GDSC). The analysis method is based on the previous reports(16).

2.5 Immune microenvironment analysis for ovarian cancer

CIBERSORT is a deconvolution algorithm which transformed normalized gene matrix into distribution pattern of immune cells. Patients were divided into ACSM3 high-expression and low-expression groups to estimate the relationship between ACSM3 and immune cells. Samples for CIBERSORT estimation results with p -value < 0.05 were included. The exploration of ACSM3 with different subtypes of immune cells in tumor microenvironment was analyzed by TIMER online tool(17). The exploration of ACSM3 with immune checkpoint inhibitor and immune stimulators were analyzed by TISIDB online tool(18).

2.6 GSEA analysis

GSEA analysis was performed by the software to show gene set enrichment analysis(12). NES (normalized enrichment score) and FDR (false discovery rate) were used to show the significance of related pathways. FDR < 0.25 were viewed as significant.

2.7 Cell culture and treatment

The ovarian cancer SKOV3 and SKOV3/DDP cells lines were purchased from Shanghai Genechem Co, Ltd. Cells were cultured in RPMI1640 culture medium with 10% FBS and 5% CO₂ at 37°C. For the rescue experiments, the PI3K/AKT signaling pathway inhibitor LY294002 was used for further analyzed.

2.8 Cell transfection

Lentiviral vectors expressing small hairpin RNA (shRNA) targeting ACSM3 were named as shRNA1-ACSM3 and shRNA2-ACSM3. The lentiviral overexpressed vector pcDNA3.1 of ACSM3 was named as Lv-ACSM3. The shRNA sequences were represented in Table S3. Thus, shRNA-ACSM3 was infected into SKOV3 cell line, and Lv-ACSM3 was infected into SKOV3-DDP cell line.

2.9 CCK8 assay

In order to see the apoptosis ability of ovarian cancer cells using cisplatin. The 1×10^4 cancer cells were seeded in 96-well plates, after 24h the cisplatin was added in the cells with the concentration of 0, 5, 10, 15 and 20ug/ml. After 48h, CCK8 assay was performed.

2.10 RNA extraction and real-time PCR assay

Total RNA was extracted though TRIZOL reagent. PrimeScript RT reagent Kit with gDNA Eraser was used to reverse mRNA into cDNA, and SYBR Premix Ex Taq was used to perform real-time PCR using ABI 7500 fast. The primer sequences were represented in Table S4. GAPDH was used as the reference mRNA.

2.11 Western Blot assay

Total protein of cells was extracted by RIPA lysate. The quantification of proteins were analyzed by BCA method. Western blot was performed by adding 40-60ug using 10% SDS-PAGE. 5% milk was used to incubate for 2h. Primary antibody was used to incubate for overnight at 4°C, including anti-ABCG2, anti-MDR1 and anti-GAPDH. And the membranes were also incubated in the secondary antibody for 2h. GAPDH was used as the reference protein.

2.12 Statistical analysis

As for assessing the independent prognostic molecules, univariate and multivariate cox proportional hazard regression analysis were used and calculated by hazard ratios (HR). Kaplan-Meier method and log-rank test were used to test overall survival, disease-free survival and progression-free survival time.

Pearson analysis was constructed to analyze the correlation between ACSM3 and other fatty acid metabolism-related genes. The differences between two groups were tested by Student t-test, p -value < 0.05 were considered statistically significant. Data was analyzed by GraphPad Prism 7 software (GraphPad Software Inc., La Jolla, CA, USA). For the bioinformatics analysis, R software was used. The differences in clinicopathologic parameters between high-risk and low-risk groups were analyzed by Chi-square Test.

3. Results

3.1 ACSM3 is down-regulated in ovarian cancer

396 fatty acid metabolism-related genes were collected from the Molecular Signature Database as the candidate genes (Table S1). Univariate cox proportional hazard regression analysis combined with Kaplan-Meier methods were used to analyze the correlations between candidate genes and prognosis of cancer. The results showed that 6 genes were collected as the favorable prognostic-related genes, including ABCD4, ACSM3, ANGPTL3, CYP3A4, GSTZ1 and NR1H3. Among them, ACSM3 acted as a tumor suppression gene in multiple cancers, especially in ovarian cancer (Fig. 1a). For further validation, the expression of ACSM3 in public datasets, the Oncomine database was used. Compared to the ovary tissues and borderline ovarian cancer tissues, the results showed that the expression of ACSM3 is significantly down-regulation in ovarian cancer tissues (Fig. 1b). Ovarian cancer patients with higher expression of ACSM3 showed better overall survival prognosis (Fig. 1c). To demonstrate the importance role of ACSM3 played in fatty acid metabolism, we analyzed the relationship between ACSM3 and other essential fatty acid metabolism-related genes by Pearson analysis, such as ACSM1, PRKAB1 and ACOX2 (Fig. 1d). The results showed that the expression of ACSM3 positively correlated with these fatty acid metabolism genes, which demonstrated ACSM3 play an essential role in fatty acid metabolism.

3.2 Analysis and validation of ACSM3 correlated with better prognosis in ovarian cancer

To validate the prognostic value of ACSM3 in ovarian cancer, two independent cohorts from GEO dataset were analyzed using the same formula. In GSE63885, which including 66 patients, the expression of ACSM3 closely correlated with the favorable overall survival and disease-free survival. Similarly, in GSE30161, which including 44 patients, the expression of ACSM3 also showed closely correlated with the favorable overall survival and progression-free survival (Fig. 2a). In the stratified cohort, patients were classified by the most important parameters, such as age, grade and clinical stages. All the cohorts showed that the group with higher expression of ACSM3 had better prognosis independent of those clinical characters (Fig. 2b). Nomogram could work as a quantitative method to predict the survival probability. Thus, we constructed a nomogram which including various clinicopathological parameters. The nomogram could estimate 1- to 5-year survival probabilities of ovarian cancer patients, which showed the expression of ACSM3 plays an important role in prediction of prognosis. To calculate the

manifestation of nomogram, C-index was used (Fig. 2c). Here, in our nomogram, the C-index was up to 0.7825, which demonstrated optimal agreement compared with the ideal model. Through Oncomine database, we further investigate the relationship between ACSM3 expression and grade, stage and recurrence status (Fig. 2d). Although we used distinct datasets to investigate the prognostic value of ACSM3 in ovarian cancer, we should further investigate whether ACSM3 could work as an independent prognostic molecule using univariate and multivariate cox proportional hazard regression analysis (Table 1). The results showed that the expression of ACSM3, age and clinical stages were acted as the independent prognostic factors. In Table 2, we investigated the relationship between ACSM3 and clinical parameters, results showed that the expression of ACSM3 significantly correlated with lymphatic invasion, KPS, venous invasion, neoplasm cancer status, race and BRCA1/2 mutation. Taken together, all these cohorts indicated that ACSM3 could work as a favorable prognostic predictor for ovarian cancer.

3.3 ACSM3 regulate PI3K/AKT signaling pathway in ovarian cancer

According to the expression of ACSM3, ovarian cancer patients were divided into high-expression group and low-expression group, and DEGs between these two groups were further investigated. PPI network was constructed based on the DEGs (Fig. 3a). Interestingly, GO and KEGG analysis was constructed, the results showed that ACSM3 significantly correlated with the immune response and PI3K/AKT signaling pathway (Fig. 3b). Therefore, we further analyzed the role of PI3K/AKT inhibitors, namely Apitolisib, in ovarian cancer cells. Through GDSC database, the IC50 values on pan-cancer cell lines of Apitolisib was predicted, the results showed that ovarian cancer cells are sensitivity to Apitolisib (Fig. 3c). The IC50 curve was shown in Fig. 3d, and most of the cancer cells are sensitivity to Apitolisib, especially ovarian cancer cells. In addition, Apitolisib also correlated with the mutation status of BRCA1, PIK3R1 and PTEN in ovarian cancer (Fig 3e). It's worth noting that patients with BRCA1 mutation, PIK3R1 mutation and PTEN mutation showed more chemosensitivity to the drugs.

3.4 ACSM3 correlated with the immune infiltration of ovarian cancer

To validate the correlation between ACSM3 and immune cells, TIMER online tools were used. The results showed that the immune status, expressed by the tumor infiltrating lymphocytes, closely correlated with the expression of ACSM3 (Fig. 4a). As a typical immunosuppressive tumor, ovarian cancer can benefit from immunotherapy. However, currently there is no mature immunotherapy for ovarian cancer in clinical application. In fact, the combination usage of drugs based on immune checkpoint inhibitor and costimulatory molecule agonists shows the future research direction⁽¹⁹⁾. Thus, the relationship between ACSM3 and immune checkpoint inhibitor, costimulatory molecule agonists were analyzed by TISIDB online tools. As for immune checkpoint inhibitors, ACSM3 significantly correlated with the expression of CSF1R, CXCL12, CD86 and HAVCR2, the receptors of macrophages (Fig. 4b). These results showed that

ACSM3 might participate in regulating the sensitivity of immune checkpoint inhibitors. To demonstrate the relationship between ACSM3 and ovarian cancer immune microenvironment, the violin plot was constructed, which showed expression of ACSM3 correlated with B cells memory, plasma cells, T cells follicular helper and activated dendritic cells in ovarian cancer (Fig. 4c). GSEA was used to investigate the pathways that ACSM3 might participate in from KEGG. Through GSEA analysis, ACSM3 correlated with the endocytosis and Fc mediated phagocytosis significantly, which these pathways showed strong correlation with immune signaling of macrophages (Fig. 4d). Based on the results, ACSM3 might influence immune microenvironment through activating those pathways, especially its close relationship with macrophages.

3.5 ACSM3 inhibited the chemoresistance for ovarian cancer

To validate the relationship between ACSM3 and chemoresistance in ovarian cancer, we investigated the correlation between ACSM3 and apoptosis-related molecules through pearson analysis, including caspase 3 and caspase 9 through GEPIA online database (Fig. 5a). CCK8 assay was used to see the expression of ACSM3 and cisplatin-resistance of ovarian cancer cell lines SKOV3 and SKOV3-DDP (Fig. 5b). The inhibition of ACSM3 in SKOV3 showed chemosensitivity to cisplatin, and the overexpression of ACSM3 in SKOV3-DDP showed chemoresistance to cisplatin. RT-PCR was used to investigate the mRNA expression of chemoresistance-related genes ABCG2 and MDR1. Inhibition of ACSM3 showed higher expression of ABCG2 and MDR1, and overexpression of ACSM3 showed lower expression of these two genes (Fig. 5c). What's more, western blot was used to validate the expression of these two genes, and showed similar results (Fig. 5d).

3.6 ACSM3 inhibited the chemoresistance through PI3K/AKT signaling pathway

LY294002 was used as the inhibitor of PI3K/AKT signaling pathway, to see whether ACSM3 can regulate the chemoresistance of ovarian cancer through PI3K/AKT signaling pathway. The groups were divided into 5 groups, including NC, shRNA1-ACSM3 group, shRNA2-ACSM3 group, shRNA1-ACSM3 plus LY294002 group and shRNA2-ACSM3 plus LY294002 group. CCK8 assay was used to see the function of inhibiting ACSM3 and LY294002 in cisplatin-resistance of ovarian cancer cell, and LY294002 can rescue the chemosensitivity of shRNA-ACSM3 function (Fig. 6a). In Fig. 6b and Fig. 6c, we used RT-PCR and western blot to investigate the expression values of chemoresistance-related genes, ABCG2 and MDR1. The results showed that inhibit ACSM3 can promote the expression of ABCG2 and MDR1, and LY294002 can reverse these overexpression of ABCG2 and MDR1.

4. Discussion

Acyl-CoA medium-chain synthetase ACSM3 is a member belongs to ACSM family, which participates into the first step of fatty acid metabolism(20). The regulation of acyl-CoA synthetase family subunits occurs in many diseases. In prostate cancer, long-chain acyl-CoA synthetases (ACSL) catalyzed fatty acids into fatty acyl-CoA, and ACSL4 could promote the progression of prostate cancer through AR-independent via ACSL4 mediated fatty acid metabolism(21). As for ACSM3, it could inhibit cancer progression through down-regulation of WNK1 and AKT phosphorylation in hepatocellular carcinoma(9). Similarly, in our study, we found that the aberrant expression of ACSM3 also influenced the progression of ovarian cancer. We validated that fatty acid metabolism-related gene ACSM3 showed favorable prognosis of ovarian cancer patients through various independent cohorts. Generally, ACSM3 worked as the anti-oncogene in pan-cancer according to its expression in normal and cancer tissues, especially in ovarian cancer. Thus, we constructed a novel nomogram including ACSM3 combined with clinical parameters to predict the prognosis for patients, which showed the expression of ACSM3 is very essential in ovarian cancer.

Nowadays, the study of basic research and clinical application of tumor immunotherapy are very hot. Many studies focused on study the mutation and aberrant expression of genes, which could influence the immune status of ovarian cancer. Shen et al. showed that in ovarian cancer mice model, ARID1A deficiency correlated with the increasing of gene mutation burden, elevating tumor-infiltrating lymphocytes and PD-L1 expression, which influenced the immune checkpoint blockade treatment(22). In addition, ACSM3 showed correlation with the Fc mediated phagocytosis signaling, which is essential in the function of macrophage phagocytosis(23). The endocytosis function of ACSM3 mediated also correlated with immune antigen presentation(24). In fact, the immunotherapy of ovarian cancer is limited to basic research and pre-clinical study. No immune treatment-related drugs have been used in clinical targeted immunotherapy in ovarian cancer. Thus, we tried to study the significance of ACSM3 in predicting the prognosis of ovarian cancer with immunotherapy. In cisplatin-resistant ovarian cancer cells, the expression of CSF-1R up-regulated, and inhibited the CSF-1R increased the cisplatin sensitivity(25). In BRCA1-deficient ovarian cancer, combined blockade of CTLA4 and PARP inhibitor, could induce protective antitumor immunity, which showed favorable prognosis for ovarian cancer(26). Here, we found that ACSM3 significantly correlated with the expression of macrophage, especially the membrane protein of macrophages.

The most importantly, we found that ACSM3 might participate in promoting the chemosensitivity of ovarian cancer cells by PI3K/AKT signaling pathway through bioinformatics, which hasn't been investigated yet. Thus the drug sensitivity analysis from GDSC database showed that the inhibitor of PI3K/AKT signaling pathway might participate in regulating chemosensitivity of ovarian cancer cells, which also validated by our in vitro study. Therefore, our study first confirmed that ACSM3 can promote the chemosensitivity of ovarian cancer through PI3K/AKT signaling pathway.

5. Conclusions

In conclusion, as a novel favorable prognostic biomarker, ACSM3 closely correlated with immune checkpoint inhibitor in ovarian cancer patients. The nomogram including the expression of ACSM3 and

those important clinicopathological parameters, showed that ACSM3 worked as a worthy addition. According to the analysis, we found that ACSM3 can promote the chemosensitivity of ovarian cancer cells through PI3K/AKT signaling pathway. This study provided a novel research direction for ovarian cancer therapy.

Abbreviations

ACSM3: acyl-CoA synthetase medium-chain family member 3; CIBERSORT: cell type identification by estimating the relative subset of known RNA transcripts; C-index: concordance index; CSF-1R: colony-stimulating factor 1 receptor; CTLA4: cytotoxic T-lymphocyte-associated protein 4; DC: dendritic cancer; FASN: fatty acid synthase; FDR: false discovery rate; GEO: gene expression omnibus; GEPIA: gene expression profiling interactive analysis; GSEA: gene set enrichment analysis; HR: hazard ratios; KEGG: the Kyoto encyclopedia of genes and genomes; MDSC: myeloid-derived suppressor cells; NES: normalized enrichment score; NK: nature killer; PPAR: peroxisome proliferator-activated receptor; TCGA: the cancer genome atlas; TIL: tumor-infiltrating lymphocytes.

Declarations

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Author's contributions:

AY and DH conceived and designed the study. AY performed the analysis, did the experiments and wrote the paper. DH revised the manuscript. All authors read and approved the final manuscript.

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Competing interests:

The authors declare that there is no potential competing interest.

Author details:

Gynecological Mini-Invasive Center, Beijing Obstetrics and Gynecology Hospital, Capital Medical University. Beijing Maternal and Child Health Care Hospital, 17 Qihelou Street, Dongcheng District, Beijing 10006, China.

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Tables

Table 1

Univariate and multivariate cox regression analysis of ACSM3 and other clinical characteristics predictive of overall survival in TCGA dataset

Genes	Univariate		Multivariate	
	HR	<i>p</i> -value	HR	<i>p</i> -value
ACSM3 expression	1.465	0.002	1.429	0.004
Age	0.760	0.024	0.701	0.004
Stage	1.509	0.001	1.497	0.001
Grade	1.375	0.044	1.248	0.168
Lymphatic invasion	1.439	0.102		
Venous invasion	0.971	0.910		

Table 2

The expression of ACSM3 with the clinic pathological parameters in ovarian cancer

Characteristics	High-risk (n=264)	Low-risk (n=263)	p-Value
<i>Age</i>			0.3793
Mean (years)	60.06±0.7198	59.17±0.7097	
<i>FIGO stage</i>			0.5294
I	9	6	
II	12	13	
III	209	197	
IV	33	45	
NA	1	2	
<i>Residual tumor size</i>			0.1055
No macroscopic	63	42	
1-10mm	117	115	
11-20mm	16	18	
>20mm	41	59	
NA	27	29	
<i>Lymphatic invasion</i>			<0.0001*
Yes	43	89	
No	43	34	
NA	178	140	
<i>KPS</i>			0.0069*
40	2	0	
60	8	7	
80	30	10	
100	2	5	
NA	222	241	
<i>Venous invasion</i>			0.0077*
Yes	31	54	
No	42	25	
NA	191	184	

<i>Therapy outcome</i>		0.1936
Complete remission	160	135
Partial remission	27	32
Progressive disease	13	23
Stable disease	15	15
NA	49	58
<i>Neoplasm cancer status</i>		0.0215*
With tumor	158	175
Tumor free	80	53
NA	26	35
<i>New neoplasm event type</i>		0.6899
Recurrence	129	134
Progression of disease	15	12
Locoregional disease	2	4
Metastatic	0	1
NA	118	112
<i>Radiation therapy</i>		0.9021
Yes	2	3
No	255	253
NA	7	7
<i>Tumor grade</i>		0.7233
G1	4	2
G2	34	32
G3	220	222
G4	1	0
NA	5	7
<i>Race</i>		0.0075*
White	218	238
Black or African American	18	5

Asian	12	7
American Indian	0	3
NA	16	10
<i>BRCA1/2 mutation</i>		0.0448*
Yes	26	36
No	107	124
NA	131	103

Figures

Figure 1

The expression of ACSM3 was down-regulated in ovarian cancer. (A) The expression of ACSM3 was down-regulated in ovarian cancer through GEPIA database;(B) The expression of ACSM3 was down-regulated in ovarian cancer through Oncomine database; (C) The expression of ACSM3 is correlated with

the favorable overall survival analysis of ovarian cancer from TCGA dataset; (D) ACSM3 positively correlated with other fatty acid metabolism-related genes, including ACSM1, PRKAB1 and ACOX2.

Figure 2

Analysis and validation the expression of ACSM3 correlated with the prognosis of ovarian cancer through independent cohorts. (A) The expression of ACSM3 correlated with the overall survival, disease-free survival and progression-free survival analysis in independent cohorts GSE63885 and GSE30161; (B) Overall survival cohorts stratified of ACSM3 in ovarian cancer through age, grade and stage; (C) The prognostic nomogram and calibration curves of nomogram were analyzed for ovarian cancer patients; (D) The expression of ACSM3 with the grade, stage and recurrence status of ovarian cancer through Oncomine database.

Figure 3

ACSM3 correlated with the chemosensitivity of ovarian cancer through PI3K/AKT signaling pathway. (A) PPI network analysis of DEGs in high-expression group and low-expression group of ACSM3; (B) GO and KEGG analysis of DEGs in high-expression group and low-expression group of ACSM3; (C) IC50 distribution of Apatolisib by tissue type; (D) The IC50 distribution of Apatolisib of all cell types and ovarian cancer cell types; (E) IC50 of Apatolisib on ovarian cancer cells with gene mutation, including BRCA1, PIK3R1 and PTEN.

Figure 4

ACSM3 closely correlated with the immune infiltration of ovarian cancer. (A) The correlation between ACSM3 and immune cells in ovarian cancer through TIMER database; (B) The correlation between ACSM3 and immune checkpoint inhibitors associated with macrophages in ovarian cancer through TISIDB database; (C) The violin plot showed the differences of 20 immune cells proportions between two groups. Blue color represented low-expression of ACSM3; red color represented high-expression of ACSM3; (D) The correlation between ACSM3 and macrophage-related pathways in ovarian cancer through GSEA analysis.

Figure 5

ACSM3 promoted the chemosensitivity of ovarian cancer. (A) The expression of ACSM3 correlated with CASP9 and CASP3 through GEPIA database; (B) ACSM3 promoted chemosensitivity of ovarian cancer through CCK-8 assay; (C) ACSM3 inhibited the mRNA expression of chemoresistance-related genes ABCG2 and MDR1; (D) ACSM3 inhibited the protein expression of chemoresistance-related genes ABCG2 and MDR1.

Figure 6

ACSM3 promoted the chemosensitivity through PI3K/AKT signaling pathway. (A) CCK8 assay demonstrated that ACSM3 promoted chemosensitivity of ovarian cancer through PI3K/AKT signaling pathway; (B) LY294002 can reverse the inhibition of mRNA expression of chemoresistance-related genes ABCG2 and MDR1 through RT-PCR when inhibiting ACSM3; (C) LY294002 can reverse the inhibition of protein expression of chemoresistance-related genes ABCG2 and MDR1 through western blot when inhibiting ACSM3.

Supplementary Files

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