

The Detection of sAMY1 α in Ovarian Cancer Ascites During First-Line Chemotherapy Predict the Response of Patients to Treatment

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Abstract

Background: Ovarian cancer ascites are a clinical conundrum in the process of diagnosis and treatment, and chemotherapy is the main treatment. The efficacy and prognostic assessment of chemotherapy has been the subject of continuous exploration.

Methods: Affymetrix HTA2.0 microarray analysis of ovarian cancer ascites without and with chemotherapy, as well as metabolomics techniques, showed that AMY1A gene expression in the chemotherapy was significantly higher than that without chemotherapy.

Results: The analysis of ovarian cancer tissues, ascitic precipitated cells and supernatants, showed that the high expression of sAMY-1 α was negatively correlated with HE4 ($p < 0.01$), and was also negatively correlated with the expression of the Beclin1 and LC3 ($p < 0.05$, $p < 0.001$) in group with chemotherapy. The results of ovarian cancer cell lines OVCAR3 and A2780, which were treated with autophagic inhibitor (3MA), inducer (rapamycin) or cisplatin (CDDP), were consistent with it. The results of 3MA group showed that as the drug concentration increased, there were decreased in the levels of Beclin1 and LC3II, and sAMY1 α levels increased. There was opposite result in rapamycin group. CDDP group revealed significantly decreased levels of LC3II, Beclin1, and increased levels of sAMY1 α ; when rapamycin was added, autophagy inhibition was alleviated, and slightly reduced levels of sAMY1 α . The results indicated that chemotherapy induced autophagic death of tumor cells, and the expression of sAMY1 α in ascites of chemotherapy reflected the degree of autophagic death of tumor cells.

Conclusions: During the diagnosis and treatment of ovarian cancer ascites, the expression level of sAMY1 α was detected at any time, compared with the levels of HE4, Beclin1 and LC3 in the ascites with chemotherapy, and compared with the serum level of CA125 in the patients, so that the recurrence of ascites could be predicted. Therefore, the adjustment of treatment regimen has important guiding significance.

Background

Ovarian cancer ascites is a common complication of advanced ovarian cancer with poor prognosis¹, and the formation of which is due to the peritoneal dissemination of cancer cells². Targeted killing of cancer cells in ascites, reducing or inhibiting the recurrence of ascites, can improve the quality of life and survival time of patients^{3,4}. The preferred clinical treatment for ovarian cancer with ascites is surgery combined with chemotherapy. However, not all patients can suppress the recurrence of ascites after chemotherapy. The reaccumulation or recurrent of ascites is the outcome which the failure of chemotherapy or drug resistance⁵. So, it is crucial to find biomarkers in ascites that can predict the prognosis of ovarian cancer ascites.

Genechip technology is a new molecular biology technology emerging in the early 1990s with the advantages of high throughput, high parallelism, high sensitivity and strong specificity⁶. Metabolomics is a fast and effective way to identify new cancer biomarkers. It integrates high-throughput and high-resolution analytical technology with bioinformatics, studies the level of biological metabolism, provides a unique perspective on understanding organisms, and has increasingly mature applications in cancer research^{7,8}. Our team performed a genechip test on ovarian cancer ascites samples, and found that AMY1A-C (salivary alpha-

amylase, sAMY) and AMY2A(pancreatic alpha-amylase) genes were highly expressed in the ascites. Then metabolomics tests were performed on samples of ovarian cancer tissues, and the obtained differential metabolite, maltotriose. Maltotriose was catalyzed by α -amylase encoding the gene AMY. The TCGA database data show that all patients with serous ovarian cancer III-IV can express AMY1A-C and AMY2A, 2B genes.

sAMY is the main component of salivary protein. It is normally expressed in salivary gland, thyroid, lung, pancreas and testis tissues, while expressed in patients with ovarian, thyroid and lung adenocarcinomas⁹. Tsunashima detected sAMY in ovarian tumor cells in 1976¹⁰. In 1978, Corlette MB et al. also detected sAMY in ovarian cancer tissue and ascites¹¹. What is the relationship between the high expression of AMY gene in ovarian cancer tissue and ascites and the prognosis of ovarian cancer patients? In particular, can it be a marker for evaluating the efficacy of chemotherapy in patients with ovarian cancer ascites? The results of this study are for reference by peer experts.

Materials And Methods

Collection of patient samples

35 samples of serous ovarian cancer tissue and 74 samples of ovarian cancer ascites were gathered from Department of Gynecology of Tumor Hospital Affiliated. Among these samples, 46 cases were treated without chemotherapy, and 28 cases were treated with chemotherapy. All of the ovarian cancer samples had complete clinical and pathological data and follow-up data.

Main of antibodies and reagents

ACK lysis (Leagene Biotechnology, China), TRIzol (Invitrogen, USA), Affymetrix HTA2.0 genechip (Affymetrix, USA), sAMY1 α McAb and Beclin1 McAb (Abcam, USA), CA125 and HE4 PcAb (Bioss China), LC3 PcAb (Elabscience, China). HE4 ELISA kits (Fujirebio Diagnostics, Sweden), CA125 ELISA kit (Beijing North Institute of Biological Technology, China), Beclin1 and LC3 ELISA kits (Elabscience, China). CA125/FITC (Biologend China).

Ovarian carcinoma cell lines and culture

Two ovarian cancer cell lines (OVCAR3 and A2780) were obtained from the Cell Bank, China Academy of Sciences (Shanghai, China). Cells were maintained in RPMI-1640 complete medium supplemented with 2 mM glutamine and 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere containing 5% CO₂. Through the previous cytotoxicity assay⁴¹, we found that the IC₅₀ of A2780 and OVCAR3 respectively was 30 μ M and 45 μ M to cisplatin. A2780 as relative sensitivity, and OVCAR3 as relative resistance.

Processing of ovarian cancer ascites

The cases of cancerous ascites were centrifuged for 10 min at 3500 rpm The supernatants of cancerous ascites were stored at -80 °C for subsequent analysis by ELISA. Then, moderate ACK lysis (Leagene

Biotechnology, China) buffer was added to the precipitates, which were collected into 1.5-mL centrifuge tubes. Subsequently, RNA, electron microscopy, light microscopy, and protein analyzes were performed.

Genechip

No chemotherapy (n=3) and chemotherapy (n=1) ovarian cancer ascites were selected. 1 mL TRIzol was added to the ascitic precipitate, and mixed evenly. The samples were sent to Beijing Kangpson Biological Company for expression profile analysis of Affymetrix HTA2.0 Genechip (Specific steps in supplementary materials).

Metabolomics techniques

Metabolite extraction from ovarian cancer tissues was performed as described by references ^{42,43}, and the abnormal metabolites were selected and mapped to KEGG database (<http://www.genome.jp/kegg/>). Specific steps in supplementary materials. AMY genes screened by genechip and metabolomics were compared with epithelial serous ovarian cancer data in TCGA (The Cancer Genome Atlas) database (<https://cancergenome.nih.gov/>).

Pathological techniques

Ovarian cancer tissues or precipitated ascites cells were immobilized with 10% formaldehyde, alcohol dehydration, routinely processed into paraffin blocks and then sectioned. The sections were then stained with H&E and observed under light microscope.

Precipitated ascites cells were fixed in 2.5% glutaraldehyde in PBS (pH 7.4), rinsed with PBS, and then fixed in 1% osmium tetroxide (pH 7.4). Samples were dehydrated in a graded series of acetone, and then embedded in Epon 812. Samples were cut into ultrathin sections, double-stained with uranyl acetate and lead citrate and examined with an electron microscope (H-7650).

Flow cytometry

After cell counts were performed, 1×10^6 cells were added to a 1.5mL tube, rinsed twice with PBS and centrifuged for 5 min at 1500 rpm. Cells were then rinsed once with staining buffer, and 1 μ L of CA125 (Bioss, bs-0091R) antibody was added (staining buffer as negative control), resulting in a 100 μ L total volume. After 30 min of incubation in the dark and fixation in 500 μ L of 1% paraformaldehyde, the detection assay was performed. This assay was repeated thrice.

Immunohistochemistry (IHC)

Section of ovarian cancer samples showed the presence of sAMY1 α (1:50), HE4 (1:200), CA125 (1:200), Beclin-1 (1:300), and LC-3 (1:100) using sABC method for 4 °C overnight. The description of the specific steps was omitted. Specific steps in supplementary materials. Image J quantifies the histological samples.

Western blot

The total protein from cell lines and precipitated ascites cells was extracted, and the protein concentration was determined by a BCA protein concentration detection kit. Equal amounts of cellular proteins were loaded into each well and resolved using 15% or 12% SDS-PAGE gels. Polyvinylidene fluoride membrane blotting was subsequently performed under standard conditions. The following primary antibodies were used against sAMY1 α (1:1000), HE4 (1:100), CA125 (1:500), Beclin1 (1:1000), and LC3 (1:500) for overnight at 4°C. The membrane was added a 1:5000 dilution of the secondary antibody. Then, the blot was developed with an ECL kit. GAPDH (1:5000) used as an internal reference.

ELISA

All ELISA kit detected in accordance with the manufacturer's instructions. The supernatants of ovarian cancer ascites were detected for sAMY1 α , HE4, CA125, Beclin1 and LC3, respectively. Samples were diluted by dilution buffer (sAMY1 α , 1:1000; HE4, 1:10; CA125, 1:4; Beclin1, original ascites; LC3, 1:50). Measurements were repeated thrice, and the average value was recorded.

Statistical analysis

Statistical analysis SPSS 17.0 software was used for the statistical analysis. One-way analysis of variance (ANOVA) was used to analyze the differences between groups. An independent t-test or u-test for differences in the mean value was used for comparison. Spearman's test was used to evaluate the associations. A $p < 0.05$ was considered significant, and values of $p < 0.01$ or $p < 0.001$ were considered statistically significant. GraphPad Prism was used for mapping and curve fitting.

Results

The content and characteristics of tumor cells in EOC ascitic precipitated cells

In without chemotherapy group, the tumor cells were clustered under light microscope; electron microscope results showed that the ratio of tumor nucleus to plasma increased and more autophagosome or autophagic vacuoles were seen in the cytoplasm; meanwhile, flow cytometry (CA125 label tumor cells) results indicated that the tumor cells content accounted for $52.75\% \pm 11.8\%$ (Fig. 1A). In the chemotherapy group, tumor cells became loose after chemotherapy; most of tumor cells appeared apoptosis in electron microscope; and the tumor cell content was reduced to $19.45\% \pm 5.96\%$ (Fig. 1B). In the cases of re-ascites after chemotherapy, the number of tumor cells increased significantly and arranged with a visible glandular structure. Tumor cells proliferate actively, cell membrane protuberance is rich, and cytoplasmic cytoskeleton is increased. Tumor cell expressed reaching $83.9\% \pm 13.4\%$ (Fig. 1C, showed in ref ¹²).

Affymetrix HTA2.0 Genechip, Metabolic Profiling and TCGA

At the beginning of the experiment, we collected 4 samples for genechip. By screening the differential genes among the 4 RNA quality control samples, it was preliminarily found that the largest differential gene was AMY (Fig. 2A). In order to further confirm the role of AMY, we conducted metabonomics and TCGA analysis. Metabonomic data analysis of ovarian cancer tissues (n=22) found that the expression of maltotriose was up-regulated but not statistically significant in group with chemotherapy. Furthermore, the comparison

between the chemosensitive (sensitive) group and the chemoresistant (resistance) group showed that maltotriose, the differential metabolite, was increased in the drug resistance group with statistical significance (Fig. 2B). Maltotriose was mapped to KEGG database and found to be involved in carbohydrate digestion and absorption, catalyzed by the amylase which encoding the gene AMY (map04973). RNAseq data were collected from 379 cases of serous ovarian cancer in TCGA database, in which AMY gene subtypes were expressed as AMY1A(173/379), AMY1B(356/379), AMY1C(60/379), AMY2A(379/379), and AMY2B(277/379) (Fig. 2C).

Expression of sAMY-1 α , HE4, CA125, Beclin-1, and LC-3 in ascitic precipitate cells

sAMY1 α was expressed in cytoplasm and membrane of tumor cells, and most of the tumor cells that treated with chemotherapy or without chemotherapy had high expression (Fig 3A). HE4, CA125, Beclin1 and LC3 were all significantly higher in group without chemotherapy than those treated with chemotherapy (Fig. 3A). Compared with the group without chemotherapy group (n=15), western blot analysis showed that sAMY-1 α ($p<0.01$) was significantly increased, and HE4 ($p<0.01$), CA125 ($p<0.05$), Beclin1 ($p<0.001$), and LC3 ($p<0.001$) were significantly decreased in the chemotherapy group (n=10), with statistical significance (Fig. 3B). In conclusion, the expression of sAMY1 α may be negatively correlated with the expression of HE4, CA125, Beclin1 and LC3.

Expression of sAMY-1 α , HE4, CA125, Beclin1, and LC3 in ovarian cancer tissues

Pathologic features of serous adenocarcinoma (Fig.4A). The expression of sAMY1 α in group without chemotherapy was lower than that with chemotherapy (Fig.4B). HE4 and CA125 were highly expressed in cancer tissues of group without chemotherapy, and expressed lower in chemotherapy group (Fig.4B). There was no significant difference of Beclin1 in ovarian cancer tissue that without and with chemotherapy (Fig.4B). The expression of LC3 was significantly higher in ovarian cancer tissues that without chemotherapy, and was significantly lower in chemotherapy group (Fig.4B). Image J quantifies the histological samples which show in Fig.4C. In conclusion, the expression of sAMY1 α in both group without and with chemotherapy showed a contrary trend to that of HE4, CA125, Beclin1 and LC3.

Correlation between the expression of sAMY1 α , HE4, CA125, Beclin1, LC3 in ovarian cancer ascites and serum CA125

ELISA detected 74 samples of ovarian cancer (46 cases were treated without chemotherapy, and 28 cases were treated with chemotherapy) ascitic supernatant and the results of boxplots indicated that the expression of sAMY1 α was significantly higher in group with chemotherapy ($p<0.001$); on the contrary, the expression of HE4 ($p<0.001$), Beclin1 ($p<0.05$), LC3 ($p<0.001$) and serum CA125 ($p<0.001$) in group with chemotherapy was significantly lower than that without chemotherapy (Fig 5A). Meanwhile, correlation analysis showed that the expression of sAMY1 α was negatively correlated with expression of HE4 ($p<0.0001$), Beclin1 ($p<0.001$), LC3 ($p<0.001$), and serum CA125 ($p<0.05$) (Fig 5B). In addition, the expression of HE4 ($p<0.0001$), Beclin1 ($p<0.001$), and LC3 ($p<0.001$) in ascites was positively correlated with serum CA125; and the expression of ascitic CA125 ($p<0.0001$) was also positively correlated with serum CA125 (Fig 5C).

Correlation between the expression of sAMY1 α , HE4, CA125, Beclin1, LC3 in ovarian cancer ascites and clinical pathological data

In the 74 cases of ovarian cancer ascites, the expression of sAMY-1 α was significantly difference in group with and without chemotherapy ($p < 0.001$). The expression of HE4 was statistically significant with chemotherapy, histological type and differentiation ($p < 0.001$). Serum CA125 was significantly difference with chemotherapy ($p < 0.05$), differentiation ($p < 0.001$), and lymph node metastasis ($p < 0.05$). The expression of ascitic CA125 had no correlation with any of these clinicopathologic data. Beclin1 was expressed lower in group with chemotherapy than that without ($p < 0.01$) and highly expressed in patients less than 50 years of age ($p < 0.01$). The expression of LC3 was statistically significant with chemotherapy and lymph node metastasis ($p < 0.001$). Show in Table 1.

Correlation among the expression of sAMY1 α , Beclin1, and LC3

The ovarian cancer cell lines OVCAR3(relative resistance) and A2780(sensitivity) were treated with autophagic inhibitor (3MA) and inducer (rapamycin). OVCAR3 and A2780 were treated with different concentration of 3MA(5mM, 7.5mM, 10mM) and rapamycin (50nM,100nM, 150nM). The results of 3MA group showed that as the drug concentration increased, there were decreased in the protein levels of LC3II and Beclin1, and sAMY1 α levels increased. There was opposite result in rapamycin group, there were increased in the protein levels of LC3II and Beclin1, and sAMY1 α levels decreased (Fig. 6A,B).

Subsequently, OVCAR3 and A2780 cells were treated with cisplatin (CDDP) (20 μ M) alone or then add rapamycin. Compared with control group, cells that were treated with CDDP revealed significantly decreased levels of LC3II, Beclin1, and increased levels of sAMY1 α ; CDDP+ rapamycin showed that autophagy inhibition was alleviated, and slightly reduced levels of sAMY1 α . (Fig. 6A,B). This finding indicates that inhibition or induction autophagy can affect the expression level of sAMY1 α . Addition, chemotherapy inhibits the process of autophagy activation and induces autophagic death of tumor cells.

Discussion

Due to the anatomical position of the ovary, early diagnosis of ovarian cancer is difficult. Once the patient has ascites, the cancer has entered the advanced stage^{3,4,13}. The clinical treatment of patients with ovarian cancer ascites generally involves ascites pumping, first-line chemotherapy, and resection of ovarian cancer. However, some patients will have ascites again. The occurrence of repeated ascites indicates that the patient's condition is more serious at this stage. At this stage, it is not only necessary to adopt corresponding treatment methods to reduce the disease, but also to find valuable markers that can predict the recurrence of ascites.

High throughput screening (HTS) technology is to obtain a large amount of information through one experiment and find valuable information from it^{14,15}. Genechip is a highly integrated intelligent gene chip that uses micro-samples for detection. It can perform simultaneous research on the obtained large-scale and high-throughput thousands of genes¹⁶. Metabolomics technology has the advantage of high throughput which can detect thousands of metabolites and screen for differential metabolites. Compared with genomics,

transcriptomics and proteomics, metabolomics is more connected with life activities¹⁷. The cancer marker test also has certain significance for the follow-up of patients after surgery. It can be judged whether the drug has a significant effect and whether to continue the examination or treatment. Therefore, metabolomics is expected to become a “weapon” for cancer screening^{18–20}. In this study, AMY1A / AMY1B and AMY2B genes were screened from ovarian cancer tissues and ascites sedimentary cells by genechip and metabolomics techniques. Comparison with TCGA database also verified that serous ovarian cancer can express AMY genes. What is the clinical significance of the expression of AMY gene in ovarian cancer tissue or ascites, especially after chemotherapy? Little is known so far.

AMY is a general term for enzymes that hydrolyze starch and glycogen. The amylase in the body can be divided into pancreatic type and salivary gland type. The former is secreted by the exocrine glands of the pancreas and released into the blood; the latter is mainly secreted by the salivary glands. Amylase activity is also found in extracts from other tissues of the human body, such as ovaries, fallopian tubes, lungs, testes, semen and breast^{21,22}. High expression of sAMY can be detected in the serum of patients with esophageal cancer, ovarian cystadenocarcinoma, hepatocellular carcinoma, prostate cancer bone metastases, lung bronchoalveolar adenocarcinoma, etc.^{23–25}. In recent years, with increasing awareness of the effectiveness and reliability of sAMY activity detection, sAMY has become a sensitive biomarker. As a new type of tumor marker, sAMY has been widely used and concerned in the auxiliary diagnosis of various malignant tumors^{26–28}.

The team selected sAMY1 α protein to verify ovarian cancer tissues, ovarian cancer ascites supernatant and precipitated cells, and ovarian cancer cell lines. It was found that the increase in sAMY1 α after chemotherapy was negatively correlated with the level of HE4 / CA125 in patients with ovarian cancer ascites, suggesting that the level of sAMY1 α and HE4 expression in the ascites can be measured during the first-line chemotherapy to predict the effect of chemotherapy. Previous studies have suggested that HE4 levels increased significantly in ascites after ovarian cancer chemotherapy. The expression level of HE4 in ovarian cancer ascites may reflect the treatment effect of ovarian cancer patients and high levels of HE4 may predict the resistance of chemotherapy and the possibility of ascites formation^{29,30}. HE4 is a relatively new tumor marker, and has been used as a monitoring index for epithelial ovarian cancer in combination with CA125. The sAMY1 α found in this study is expected to be used as a guideline for clinical diagnosis and treatment of patients with ovarian cancer ascites during the first-line chemotherapy treatment.

Autophagy plays a dual role in promoting and inhibiting the apoptosis of tumor cells^{31–33}. Autophagy is conducive to maintaining the homeostasis of cells. During stress, cell autophagy prevents the accumulation of toxic or carcinogenic damage proteins and organelles, and inhibits the cell from becoming cancerous; however, once tumors form, cell autophagy provides cancer cells with richer nutrition and promotes tumor growth³⁴. The previous research results of the team concluded that autophagy has always been highly expressed to continuously provides nutrition for tumor cells, leading to recurrence or occurrence repeatedly of ovarian cancer ascites after chemotherapy, and also one of the causes of drug resistance¹². It is known that there are 60 enzymes and many metabolites in lysosomes, among which sialidase is one³⁵. The increase of sAMY1 α in ovarian cancer ascites after chemotherapy is presumably due to the killing effect after chemotherapy, which caused lysosome collapse to release the enzyme during the process of autophagic cell

death of tumor cells. In vitro, cytology further confirmed that inhibition or induction of autophagy in ovarian cancer cells can affect sAMY1 α expression. Chemotherapy not only inhibits autophagy activation, but also induces autophagic cell death of tumor cell. Previous research by our team also confirmed that the effects of autophagy inhibitors 3MA and rapamycin on ovarian cancer cells OVCAR3 and A2780 have shown that autophagy inhibitors and chemotherapeutics can increase the sensitivity of ovarian cancer cells to drugs to promote tumor cell death.

The production of cancerous ascites is a very difficult practical problem for the treatment of this patient, which is an indisputable fact. How to predict the recurrence of ascites due to chemotherapy resistance in advance. There are many markers currently used for the diagnosis of ovarian cancer, such as CA125, HE4, CEA, CA199, KLK6 and so on^{36,37}. HE4 is a newly proposed tumor marker. It was a new and promising ovarian cancer marker first discovered in the early 1990s and can be used as an independent indicator to predict the prognosis of ovarian cancer^{38,39}. CA125 has been proposed as a specific marker for the diagnosis of ovarian cancer as early as the early 1980s. However, CA125 has recently been found to have high sensitivity and poor specificity in the detection of ovarian cancer, with some false positives⁴⁰.

After ovarian cancer ascites chemotherapy, sAMY1 α increases and Beclin1 and LC3 levels decrease, which can accelerate the formation of autophagosomes into autophagolysosomes. The lysosomes release AMY into the ascites during degradation, which can be used as a marker for autophagy death of cancer cells in ascites. Patients with ovarian cancer ascites follow the detection of sAMY1 α levels in ascites during the first-line chemotherapy, combined with the expression level of HE4 and CA125 in the patients' serum, which provides important reference value judge the efficacy of the treatment of patients with ovarian cancer ascites, monitor drug resistance, modify the treatment plan, etc.

Declarations

Availability of data and materials

All data generated or analysed during this study are included in this published article and its additional information files.

Ethics approval and consent to participate

This study was approved by the ethics committee of The Third Affiliated Hospital of Harbin Medical University, and wrote informed consent was obtained from all patients. This study was approved by the ethics committee of Harbin Medical University.

Consent for publication

Not applicable.

Conflicts of Interest

The authors declare that they have no competing interests.

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Author Contributions

X.-M. Jin, L. Zhang designed research; D. Kong, S. Zhao, H.-X. Lv, and Y.-L. Xing collected samples and clinical-pathological data; Y. Liu, J.-Y. Li, J. Tang, and C. Qiao performed experiments; M. Liu, S. Jin performed metabolomics techniques analysis; L. Zhang, D. Kong interpreted data; Y. Liu, D.-Y. Liu analyzed data; D. Kong, Y. Liu, L. Zhang, X.-M. Jin wrote the paper. All authors read and approved the final manuscript.

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Tables

Table 1
Correlation between the expression of sAMY-1 α , HE4, CA125, Beclin1, and LC3 in ovarian cancer ascites

	n = 74(%)	sAMY1 α (OD450nm)	Ascitic HE4 (pM)	Serum CA125 (U/mL)	Ascitic CA125 (U/mL)	Beclin1 (ng/mL)	LC3 (ng/mL)
Age							
≤50	21(28.38)	0.71	2806.53	783.88	1007.21	3.09**	44.2
>50	53(71.62)	0.77	1907.43	744.63	995.59	1.04	39.15
Chemotherapy							
Without	46(62.16)	0.53	2929.56	939.02*	959.92	2.00**	58.63***
With	28(37.84)	1.12***	902.56***	454.71	1062.9	1.00	10.93
Histological type							
Serous	60(81.08)	0.76	2017.54***	726.45	1006.3	1.58	41.18
Others	14(18.92)	0.72	2784.19	881.41	967.12	1.80	38.01
Differentiation degree							
Well & moderate	24(32.43)	0.72	2453.48	447.47	847.61	1.73	41.51
Poor	50(67.57)	0.77	2022.92***	903.75***	1071.5	1.57	40.14
Lymph node metastasis							
Without	57(77.03)	0.78	2197.83	826.5	969.03	1.64	46.36
With	17(22.97)	0.66	2044.36	518.61*	1098.99	1.56	21.20***
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$							

Figures

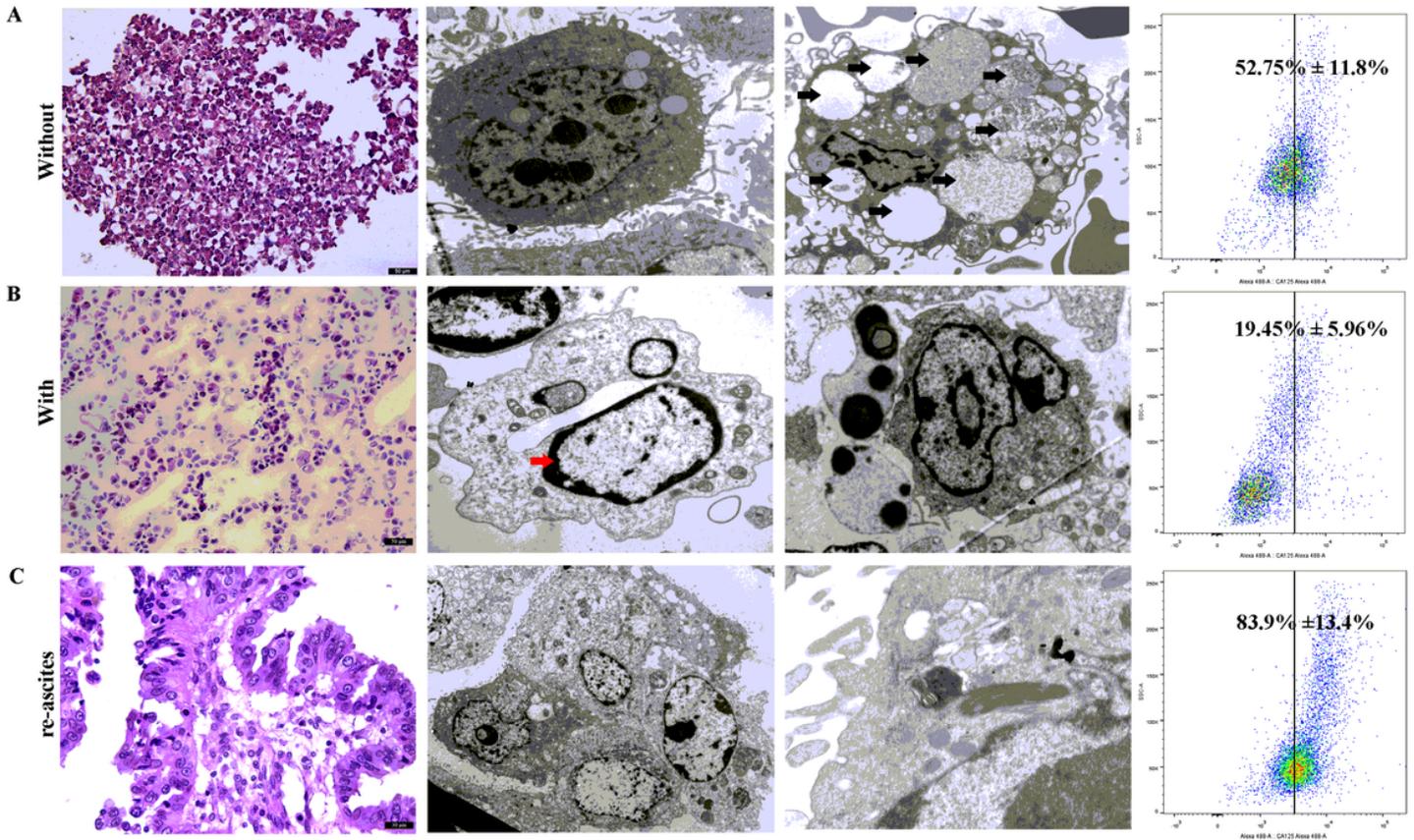


Figure 1

Identification of ovarian cancer ascitic precipitate cells. (A). In group without chemotherapy, more autophagic vacuoles or autophagosomes (arrow) were seen under electron microscope, EM×5000. CA125 was expressed as $52.75\% \pm 11.8\%$ (n=8). (B). In chemotherapy group, most of the tumor cells appeared apoptotic changes (arrow in red), EM×5000. The expression of CA125 was reduced to $19.45\% \pm 5.96\%$ (n=4). (C). In re-ascites group, tumor cell cytoplasmic cytoskeleton is increased, EM×5000. CA125 was expressed as $83.9\% \pm 13.4\%$ (n=2).

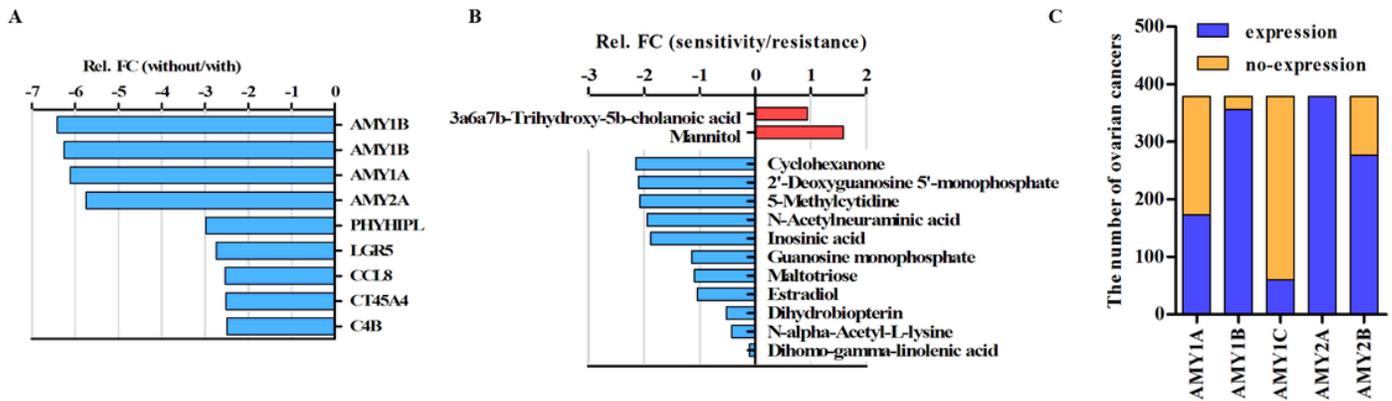


Figure 2

The AMY gene in ovarian cancer. (A). Top 9 down-regulated genes (without vs. with) after genechip analysis. (B). Metabonomic data analysis of up- and down-regulated Metabolite (sensitivity/resistance). (C). The expression quantity of AMY gene in TCGA.

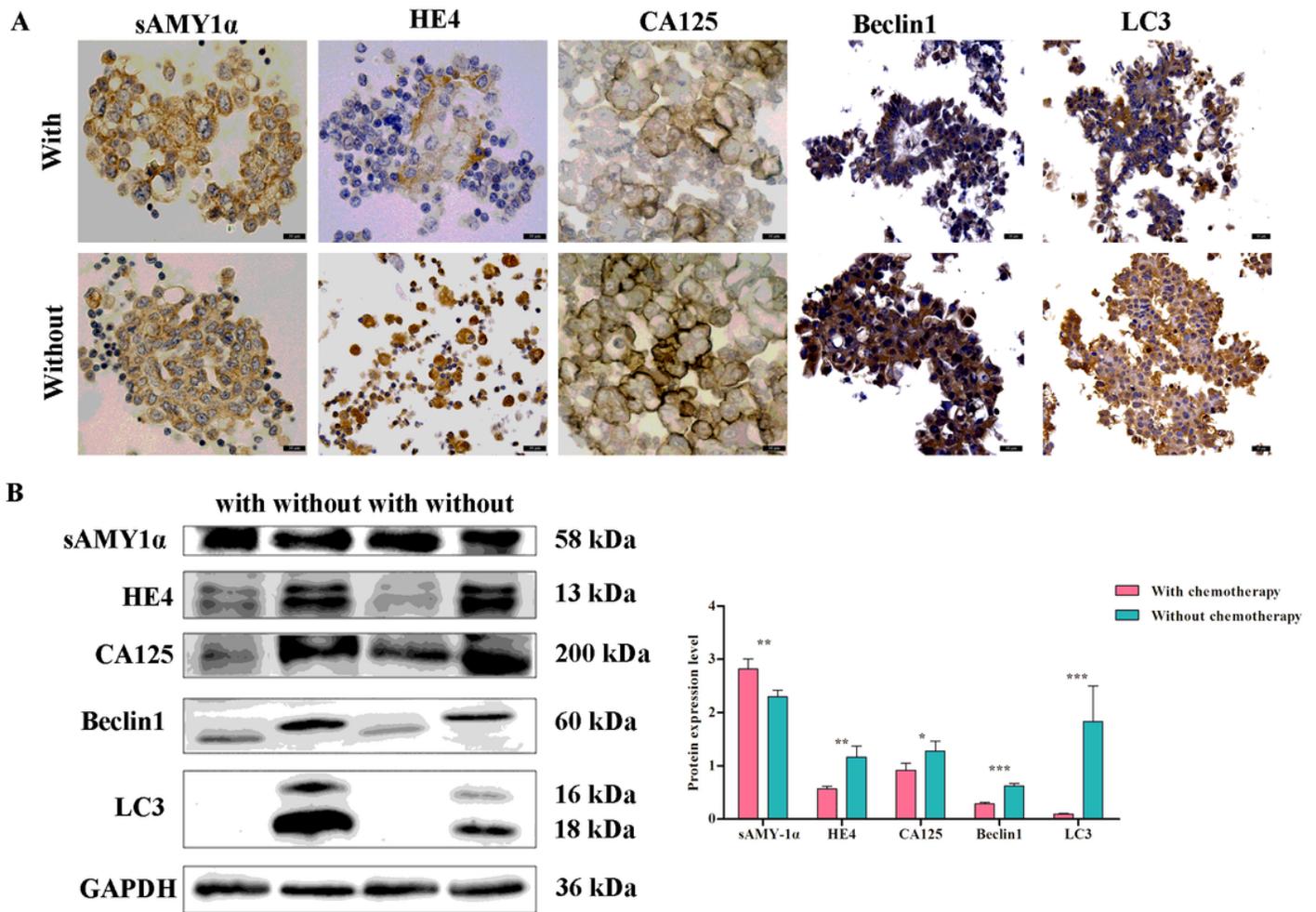


Figure 3

The expression of sAMY1 α , HE4, CA125, Beclin1, and LC3 in EOC ascitic cells. (A) sAMY1 α expressed in group with and without chemotherapy were high. HE4, CA125, Beclin1, LC3 expressed lower in group with chemotherapy than that without chemotherapy. (scale=30 μ m) (B) Four representative samples are shown. Western blot analyze the expression of sAMY1 α , HE4, CA125, Beclin1, and LC3 (n=25, *p<0.05, **p<0.01, ***p<0.001).

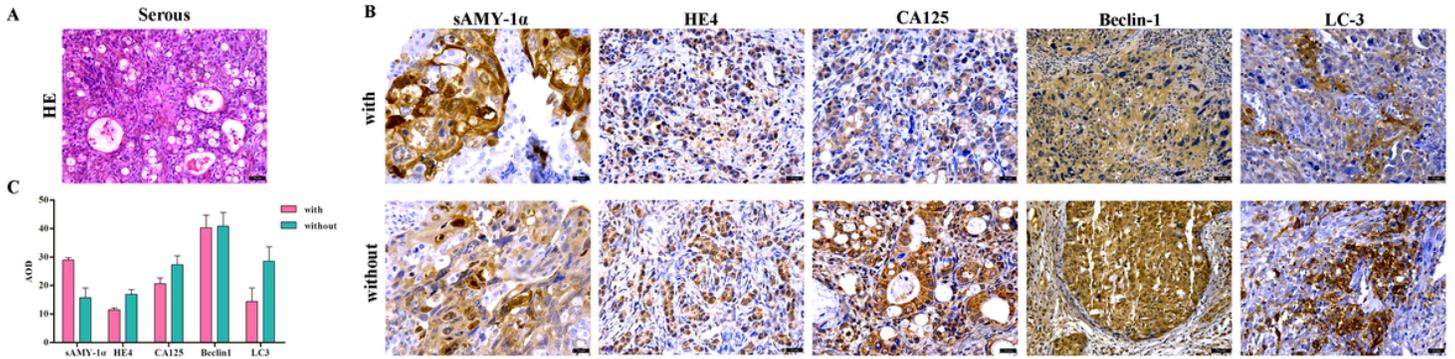


Figure 4

The expression of sAMY-1 α , HE4, CA125, Beclin-1, and LC-3 in ovarian cancer tissues. (A) Serous ovarian adenocarcinoma (scale=50 μ m). (B) The expression of sAMY-1 α , HE4, CA125, Beclin1 and LC3 in group without chemotherapy and group with chemotherapy (scale=30 μ m). (C) The quantitative results of IHC.

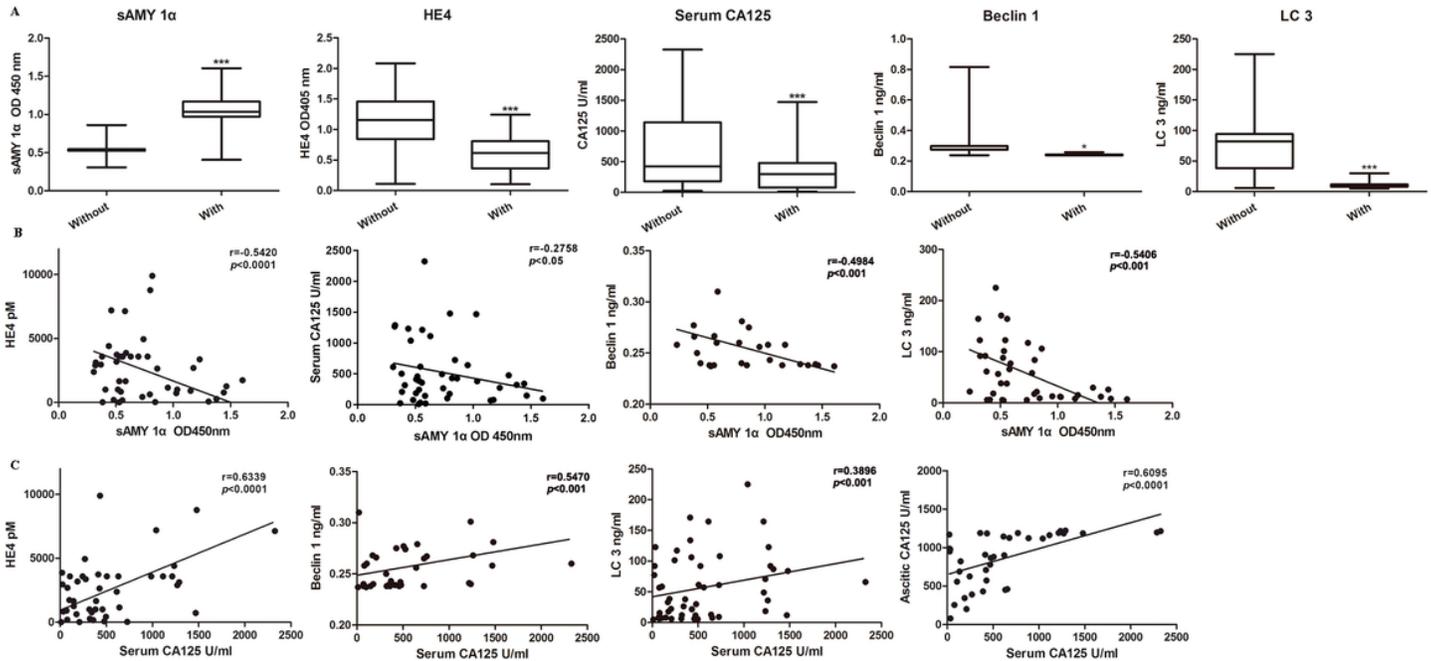


Figure 5

The correlation of sAMY1 α , HE4, Beclin1, and LC3 in ovarian cancer ascites and serum CA125. (A) Comparison of sAMY-1 α , HE4, CA125, Beclin1, and LC3 in ovarian cancerous ascites group without and with chemotherapy by ELISA. (B) The correlation between sAMY-1 α and HE4, serum CA125, Beclin1, LC3 in ovarian cancer ascites. (C) The correlation among serum CA125 and HE4, Beclin1, LC3, and ascitic CA125 in ovarian cancer. (*p<0.05, **p<0.01, ***p<0.001)

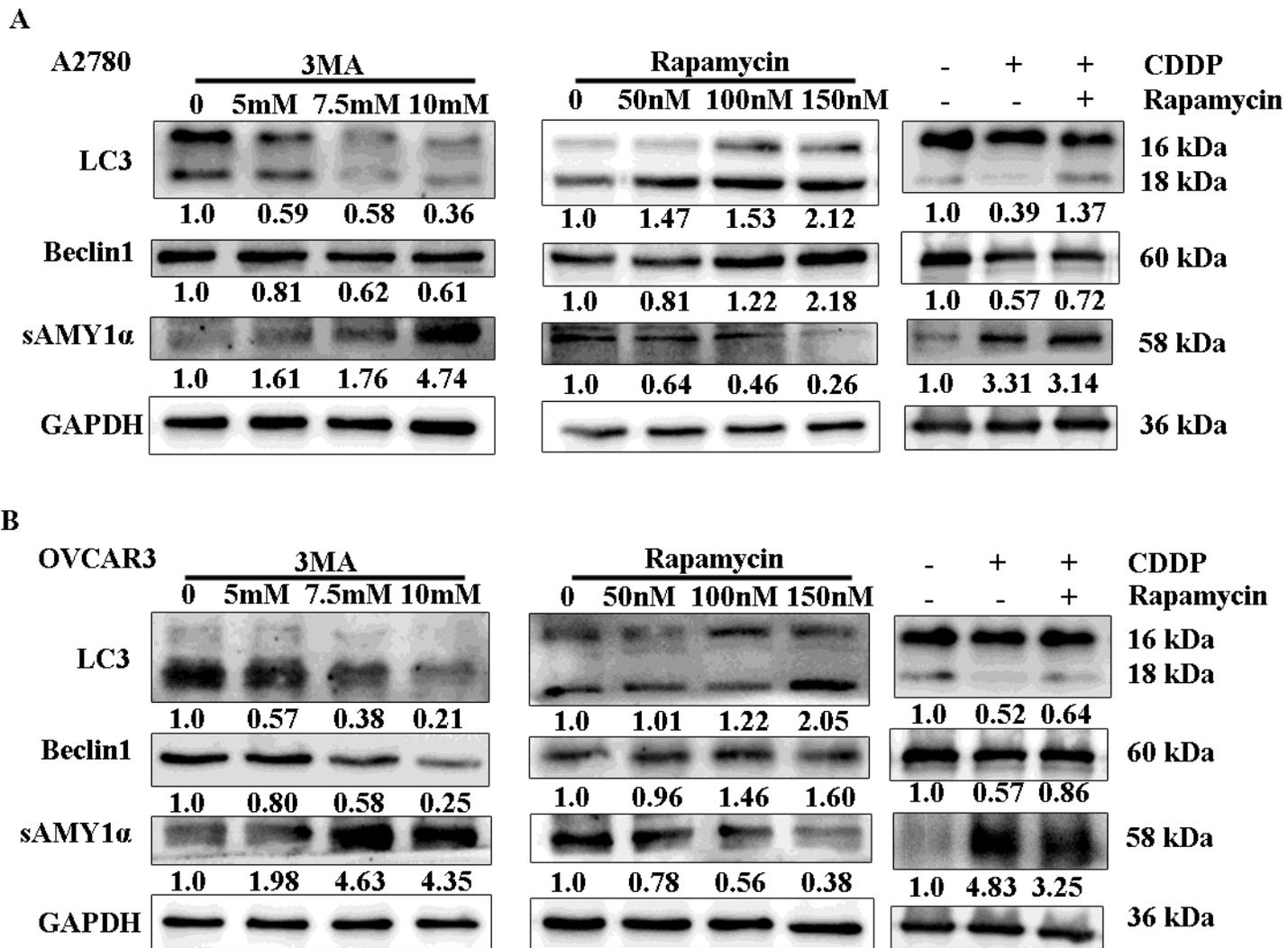


Figure 6

Correlation among the expression of sAMY1α, Beclin1, and LC3. (A). Different concentration of autophagic inhibitor, inducer and CDDP treated with A2780. (B). Different concentration of autophagic inhibitor, inducer and CDDP treated with OVCAR3.

Supplementary Files

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