Serum amyloid a, a potential biomarker both in serum and tissue, correlates with ovarian cancer progression

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Abstract

Objective: Ovarian cancer is the most fatal gynecologic malignancy worldwide due to delayed diagnosis as well as recurrence and drug resistance. Thus, a new type of ovarian cancer treatment prediction biomarker is urgently needed to supplement existing tools.

Methods: One hundred patients operated on due to ovarian tumor were enrolled in this study. Meanwhile, one hundred ovarian benign patients and thirty healthy women were selected as control groups. Levels of SAA, CA125 and HE4 were assessed using standard laboratory protocols. 5 ovarian cancer tissues and paracancerous tissues were collected and than stored at -80°C until the qRT-PCR assay was conducted.

Results: The ROC curve of SAA concentration in ovarian cancer was plotted to obtain the area under the curve AUC = 0.889, the cut-off value 17.05 mg/L, the sensitivity 78.4% and specificity 86.5%. The results by logistic regression analysis revealed that there was significant correlation between the level of serum SAA and clinical stage and lymph node and distant metastasis. Compared with pre-treatment, the level of serum SAA decreased significantly after treatment. qRT-PCR assay revealed that the mRNA of SAA-1 and SAA-4 was much higher in cancer tissues than in adjacent tissues, and MMPs was up-regulation including MMP-1, MMP-9 and MMP-12 in OVCAR-3 cell stimulated by SAA and transwell assay revealed that SAA could promote OVCAR-3 cell migration. Moreover, SAA can regulate EMT markers and promote Akt pathway activation.

Conclusion: In summary, our results demonstrated that SAA may be a potential diagnosis and treatment prediction biomarker. SAA promote OVCAR-3 cell migration by regulating MMPs and EMT which may correlate with Akt pathway activation.

Background

Ovarian cancer is a clinically common malignant tumor for women, and morbidity have reached to 6.31/10 million while the mortality rate was approximately 2.73/10 million[1]. Because the patient's early symptoms are insignificant, approximately 60–70% of patients have progressed to stage III-IV or have developed abdominal metastases[2]. Although surgical techniques and methods of radiotherapy and chemotherapy for ovarian cancer continue to advance, the 5-year survival rate for the disease is
still as low as 30%, and the prognosis is poor[3]. However, it is reported that the patients with early stage (FIGO I and II) have a better prognosis than patients with advantage stage (FIGO III and IV)[4]. Therefor, early diagnosis of ovarian cancer is extremely important for the patient's prognosis. Now, the serum markers of ovarian cancer mainly include CA125 and HE4. However, CA125 detection is poorly performed in the diagnosis of patients with early ovarian cancer[5]. The study has reported that the sensitivity and specificity of CA125 are 0.796 and 0.825[6]. Although HE4 has greater specificity than CA125, but the sensitivity has a varying results[6–8]. Thus, it is important to look for a new biomarker in the serum that can help diagnosing and predicting ovarian cancer. Previous study has revealed that Acute phase serum amyloid A in ovarian cancer as an important component of Proteome diagnostic profiling[9]. Therefore, we explored whether serum amyloid A could be a potential biomarker for ovarian cancer.

Serum amyloid A (SAA), an acute-phase protein, is mainly synthesized in the liver, dramatically increasing during inflammatory diseases [10]. The level of serum SAA can elevate more than 1000 folds during inflammation [11]. Therefore, SAA has been long considered as a sensitive marker of inflammation [12]. More and more evidence proves that chronic infection and inflammation especially bio-synthesis and secretion of pro-inflammatory cytokines is closely connected with cancer [13]. As previously reported, SAA is involved in neoplastic progress[14]. Moreover, it is reported that the concentration of SAA is significantly high in various cancer including lung cancer[15], breast cancer[16], uterine cervical cancer[17], renal cancer[18], gastric cancer[19] and others[20, 21]. The role of SAA in cancer is not very clear. Some studies have proved that SAA could influence carcinogenesis by activating the transcription factor nuclear factor kappa-B (NF-κB)[22, 23]. SAA may also favor tumor development by limiting immune anti-tumor via stimulating growth of regulatory T cells in a process involving IL-1β and IL-6 induction in monocyte[24]. Previous studies demonstrated that SAA was involved in adhesion, migration, and tissue infiltration of inflammatory cells[25], induced matrix metalloproteinases (MMPs), a family of extracellular, zinc-dependent matrix degrading proteases, including MMP-1, MMP-3[26] and MMP-9[27], which could interact with degrade extracellular matrix (ECM) controlling the diffusion and migration of cells. Several studies
demonstrated the repressing MMP-9 could inhibit the migration of cancer cell[28, 29]. Some studies have proved that SAA can induce MMPs expression[26, 30]. Possible mechanisms of SAA stimulating MMP-9 might via formyl peptide receptor like-1-mediated signaling[31]. However, whether SAA promotes ovarian cancer cell metastasis by inducing MMPs expression is not reported and there is rarely report about the value of SAA for the diagnosis, follow-up and prognosis of ovarian cancer in the open literature. .

In this study, we investigated the expression of SAA in ovarian tumor tissue and normal tissue and the relationship between SAA and prognosis of ovarian cancer patients. Furthermore, we proved the advantage of SAA in diagnosing ovarian cancer combinations of CA125 and HE4 and that SAA could be a potential biomarker. In addition, we also supplied a potential explanation that SAA promotes OVCAR-3 migration by inducing MMPs expression.

**Results**

**Serum Amyloid A (SAA) is overexpressed in ovarian cancer**

Through analysis of SAA expression in oncomine profiles from ovarian cancer patients, we found that it is overexpression in ovarian cancer samples (109 cases) compared with adjacent normal tissue samples (37 cases) (P < 0.001, Figure 1A). We further analyzed the expression of SAA in a total of 37 paired ovarian tumor tissues in this datasets and found that it was significantly upregulated in 32 of the ovarian tumor tissues compared with their adjacent normal tissues (P < 0.001) (Figure 1B). Also in this oncomine datasets, we use Kaplan-Meier survival curves demonstrated that the overall survival of patients with high expression of SAA was significantly shorter than those with low SAA expression (Figure 1C, P < 0.001).

**Overexpression of SAA is associated with advanced clinical features in ovarian cancer**

A total of 100 patients from the entire cohort were included for analyses. Details of patient characteristics are shown in Table 1. We further analyzed the association between SAA and the clinicopathological characteristics of ovarian cancer. There was no significant association between SAA expression and age, pathological differentiation and tumor site. However, high SAA expression was significantly associated with advanced FIGO stage (P = 0.003), Lymphatic invasion (P = 0.031) and
Distant metastasis (P < 0.001) (Table 2 and Table 3).

**Serum SAA levels in patients with ovarian cancer after treatment**

In order to estimate the effect of SAA in the treatment of ovarian cancer, we collected 20 patients serum after treatment because of other patients loss follow-up in all of 100 ovarian cancer patients. Strikingly, compared with pretreatment, the level of serum SAA decreased significantly after treatment (Figure 2A). Clinically, the result prompts that doctors can assess the effect of treatment for ovarian cancer patients by detected the level of serum SAA.

**SAA as a prognostic biomarker in ovarian cancer**

We examined the concentration of serum SAA, CA125 and HE4 in health, ovarian benign disease and ovarian cancer, as the Table 4 shown. Compared with healthy group, the concentration of SAA in ovarian cancer group was significantly higher and the difference was statistically significant(P<0.001) Meanwhile, there was significantly different between benign ovarian disease group and ovarian cancer group(P<0.01)(Figure 2B). There was significantly associated with advanced FIGO stage, Compared with healthy group, the concentration of SAA in FIGO III-IV group was significantly higher and the difference was statistically significant(P<0.001). ALSO,FIGO I-II group was difference with healthy group((P<0.01) (Figure 2C).

The ROC curve of SAA, CA125 and HE4 was shown as follows (Figure2D). As the Table5 shown, the ROC curve of SAA concentration in ovarian cancer was plotting to obtain the area under the curve AUC=0.889, the cut-off value 17.05 mg/L, the sensitivity 78.4% and specificity 86.5%. The ROC curve for the diagnosis of ovarian cancer with CA125 concentration was plotted. The area under the curve AUC was 0.868, the cut-off value was 245.30 mg/L, the sensitivity was 81.1%, and the specificity was 91.9%. The ROC curve of HE4 for diagnosis of ovarian cancer showed that AUC=0.917, the cut-off value at this time was 98.01 mg/L, the sensitivity was 86.5%, and the specificity was 83.8%. While combined detection of SAA, CA125 and HE4, the area under the curve (AUC) was 0.945, the sensitivity was 89.2%, and the specificity was 97.3%.. These data suggest that SAA can be used as potential biomarker and combined detection of SAA, CA125 and HE4 shows a good value for diagnosis of ovarian cancer.
SAA modulates migration of ovarian cancer cells

By determining SAA expression via gene set enrichment analysis (GSEA) [40,41] the Cancer Genome Atlas (TCGA) profiles, we found that SAA levels were positively correlated with the proliferation by affecting genes in cell-cycle regulation. Firstly, we examined the expression of SAA-1, SAA-2 and SAA-4 in ovarian tumor tissues and OVCAR-3 cell. It was found that SAA-1 and SAA-4 could be expressed in ovarian tumor tissues and OVCAR-3 cell, but not SAA-2 (Figure 3A). These results were consistent with previous study[33]. Then, we examined the mRNA expression of SAA in ovarian cancer tissues and adjacent tissues. It showed that the mRNA of SAA-1 and SAA-4 was much higher in cancer tissues than in adjacent tissues by qRT-PCR (Figure 3B). Migration assays revealed that overexpression of SAA significantly increased the OVCAR-3 cell numbers, which were approximately 1.3-fold higher at day 5 after treated with 10μg/ml SAA compared to 0μg/ml SAA(Figure.4A).Thus we focused on the MMPs. As expected, after treated OVCAR-3 cell with 10 μg/ml SAA 24h, the mRNA expression of MMP-1, MMP-9 and MMP-12 (Figure 4B) are higher than the control one. Our results demonstrate that SAA not only induce MMP-9 but also induces the expression of MMP-1 and MMP-12.

AKT signaling pathway is regulated by SAA

we next explored the role of SAA in metastasis. Epithelial-mesenchymal transition (EMT) enables tumor cells to gain invasion and migration capabilities[38], and this phenomenon has been reported in a variety of malignancies. Both qPCR (Figure.4C) and western (Figure.4D) results show that SAA can prompt mesenchymal markers N-cadherin, Vimentin and Snail up-regulation and suppress epithelial marker E-cadherin expression. However, the regulation of EMT by SAA is not clear. Because Akt pathway play an very important role in EMT[39], we explore whether SAA can influence Akt pathway. Our results demonstrate that SAA can induce the phosphorylation of AKT on Ser473 and Thr308, although the total AKT not changed (Figure.4E).These data suggest that SAA promotes OVCAR-3 cell migration by stimulating MMPs up-regulation and promoting EMT which may correlate with Akt pathway activation.

Discussion

This is the first study to demonstrate that SAA over-expression is associated with poor prognosis in
ovarian cancer patients. Multivariate analysis revealed that high SAA expression was significantly associated with unfavorable clinical features such as advanced FIGO stage \[\text{Lymphatic invasion and Distant metastasis. More importantly, high SAA expression might be an independent prognostic indicator of survival in ovarian cancer patients.}

In clinical practice, the prediction of tumor progression after treatment is of great important. The ideal prognostic marker can provide a basis for evaluating the clinical outcome, which helps clinicians choose the best treatment strategy for patients to avoid over-treatment or undertreatment. Our present study, strikingly, compared with pretreatment, the level of serum SAA decreased significantly after treatment.

we demonstrated that SAA was upregulated in ovarian cancer cells and tissues. Also it showed that the expression level of SAA protein in histological sections was significantly correlated with clinical characteristics and reduced survival time of ovarian cancer patients.

AKT is a significant mediator of the cell cycle, is usually highly activated by its phosphorylation at both the Thr308 and Ser473 sites. In human cancers, which promotes cancer cell proliferation and migration as well as provides resistance against apoptosis [42, 43]. More importantly, p-AKT is a crucial modulator of glucose metabolism in different cells [44,45]. In addition, we found that SAA promotes OVCAR-3 cell migration by stimulating MMPs up-regulation and promoting EMT which may correlate with Akt pathway activation.

However, the specific mechanism of SAA's regulation of Akt pathway activation needs further study.

Conclusions
In conclusion, SAA may serve as a useful biomarker of poor prognostic. Clinical diagnosis combine with SAA expression may help to assess therapeutic outcomes.

Methods
Patients and Samples
Blood samples were collected using a standardized procedure. After obtaining patient approval, serum samples were collected before initial surgery from 200 patients including 100 samples from patients with average age 58.81±8.7 years with ovarian cancer and 100 samples from patients with
average age 53.78±16.69 years with ovarian benign disease. All patients were hospitalized at Tianjin Medical University Cancer Hospital in China between 2017 and 2019. Patients with rheumatoid arthritis, acute inflammations infection were excluded from this study. Samples from 30 healthy age-matched women with average age 56.07±13.93 years were used as the normal controls. The results of one-way ANOVA showed that there was no statistical difference between the three groups of subjects (P=0.395). Venous blood samples were collected in pyrogen-free tubes, allowed to clot at 4°C for 1 h, and then centrifuged at 2,000×g for 10 min. The upper serum layers were carefully obtained and divided into separate vials where they were stored at -20°C until the assay was conducted. 5 ovarian cancer tissues and paracancerous tissues were collected and than stored at -80°C until the assay was conducted.

**Latex enhanced immunoturbidimetric assay of SAA**

The serum level of SAA was determined using a commercial Kit For Serum amyloid A protein Assay (Ningbo Purebio Biotechnology Co, Ltd) and Automatic Analyzer H7180ID according to the manufacturer's instructions. If the concentration of the sample exceeds the linear range, dilute it with normal saline and re-measure it.

**Cell cultures**

Ovarian cancer cell line, OVCAR-3 were obtained from the Type Culture Collection of Chinese Academy of Sciences and maintained in culture according to their recommendations.

**In vitro migration assay**

For the Transwell migration assay, OVCAR-3 cells (2×10^5/well) in 200μl of serum-free media (RPMI DMEM) were placed in the upper chamber (Corning, Cambridge, USA) of each insert with Apo-SAA (Sigma-Aldrich, USA) (0 and 10μg/ml). Next, medium supplemented with 20% fetal bovine serum (600 μl) was added to the lower chambers. After 12 hours of incubation at 37°C, the upper surface of the membrane was wiped with a cotton tip, and the cells attached to the lower surface were stained for 15 min with crystal violet. The assays were performed in triplicate.

**RNA isolation and qRT-PCR assay**

Total RNA was isolated from fresh tissue samples using the Total RNA Extraction Kit (Solarbio Science
Technology CO. Ltd, Beijing, China) according to the manufacturer’s instructions. Total RNA (2μg) was used for the synthesis of first-strand cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher, USA) under the conditions recommended by the supplier. For qRT-PCR, the SYBR green mix (Applied Biosystems) was used to run on PCR on a LightCycler 96 System (Roche, Germany). The data were displayed as 2-ΔΔCt values with GAPDH as the control. Sequences of the RT-PCR primers were shown in supplemental table 1.

**Protein extraction and western blot analysis**

Briefly, for tissue protein extraction, the OVCAR3 cells were homogenized in ice-cold RIPA (radioimmunoprecipitation assay) lysis buffer. The cell lysates were incubated in ice for 30 min followed by centrifugation at 12,000 rpm for 10 minutes. The protein concentration of cells extracts were determined using the BCA Protein Assay Kit (Pierce). For western blot, the protein (20μg) were loaded onto 12 % polyacrylamide-SDS gradient gels, and then transferred to a PVDF membrane. The PVDF membrane were blocked with 1 % BSA. The antibodies E-cadherin (Cell Signaling), Vimentin (Cell Signaling), snail (Cell Signaling), N-cadherin (Cell Signaling), β-actin (Sigma Aldrich), total AKT (Cell Signaling), p-AKT (Ser473) (Cell Signaling), p-AKT (Thr308) (Cell Signaling), were applied for protein detection.

**Statistical analysis**

SPSS 17.0 and GraphPad Prism 7 software was used for statistical analysis. One-way ANOVA was used to analyze the years of different groups (Healthy group, Ovarian benign disease group and Ovarian cancer group). Kruskal-Wallis H test was used to test the differences in concentrations of SAA, CA125, and HE4 between the three groups, and differences between certain groups are indicated by p-values calculated by the Mann-Whitney U-test. Spearman's analysis of the correlation between SAA and CA125 and HE4, respectively. ROC curve was used to test the diagnostic efficacy of SAA in ovarian cancer. Multivariate Logistic Regression Analysis was used to examine the influencing factors of SAA in patients with ovarian cancer. For all tests, P<0.05 was considered to indicate a statistically significant difference.

**Declarations**
Ethics approval and consent to participate: Study has been granted Tianjin Medical University Cancer Institute and Hospital ethics committee approval prior to commencing. All participants have informed consent.

Consent for publication: Not applicable

Availability of data and materials: All data generated or analysed during this study are included in this published article.

Competing interests: The authors declare that they have no competing interests

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ZeLi analyzed and interpreted the patient data and Datesets profiles regarding the ovarian disease, was a major contributor in writing the manuscript. Yongwang Hou performed the histological examination of the ovarian tissue. Li Ren was contributed to design of the work.

Each author was substantial contributed to the collected of blood samples. AND to have approved the submitted version (and any substantially modified version that involves the author's contribution to the study);AND to have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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Abbreviations

List of abbreviations
SAA: Serum amyloid A
CA125: Carbohydrate antigen 125
HE4: Human epididymis secretes protein
ROC: Receiver operating characteristic curve
AUC: Area under curve
FIGO: International Federation of Gynecology and Obstetrics
MMP: Matrix metalloproteinase
EMT: Epithelial Mesenchymal transition
NF-κB: Nuclear factor kappa-B
ECM: Extracellular matrix

References
[1] Siegel RL, Miller KD and Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017, 67: 7-30.
[2] Reed N, Millan D, Verheijen R and Castiglione M. Non-epithelial ovarian cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. ANN ONCOL 2010, 21 Suppl 5: v31-v36.
[3] Ozols RF, Bookman MA, Connolly DC, Daly MB, Godwin AK, Schilder RJ, Xu X and Hamilton TC. Focus on epithelial ovarian cancer. CANCER CELL 2004, 5: 19-24.
[4] Narod S. Can advanced-stage ovarian cancer be cured? NAT REV CLIN ONCOL 2016, 13: 255-261.
[5] van Haaften-Day C, Shen Y, Xu F, Yu Y, Berchuck A, Havrilesky LJ, de Bruijn HW, van der Zee AG, Bast RJ and Hacker NF. OVX1, macrophage-colony stimulating factor, and CA-125-II as tumor markers for epithelial ovarian carcinoma: a critical appraisal. CANCER-AM CANCER SOC 2001, 92: 2837-2844.
[6] Dayyani F, Uhlig S, Colson B, Simon K, Rolny V, Morgenstern D and Schlumbrecht M. Diagnostic Performance of Risk of Ovarian Malignancy Algorithm Against CA125 and HE4 in Connection With Ovarian Cancer: A Meta-analysis. INT J GYNECOL CANCER 2016, 26: 1586-1593.
[7] Chan KK, Chen CA, Nam JH, Ochiai K, Wilailak S, Choon AT, Sabaratnam S, Hebbar S, Sickan J, Schodin BA and Sumpaico WW. The use of HE4 in the prediction of ovarian cancer in Asian women with a pelvic mass. GYNECOL ONCOL 2013, 128: 239-244.
[8] Van Gorp T, Cadron I, Despierre E, Daemen A, Leunen K, Amoen F, Timmerman D, De Moor B and Vergote I. HE4 and CA125 as a diagnostic test in ovarian cancer: prospective validation of the Risk of Ovarian Malignancy Algorithm. Br J Cancer 2011, 104: 863-870.
[9] Moshkovskii SA, Vlasova MA, Pyatnitskiy MA, Tikhonova OV, Safarova MR, Makarov OV and Archakov AI. Acute phase serum amyloid A in ovarian cancer as an important component of proteome diagnostic profiling. Proteomics Clin Appl 2007, 1: 107-117.
[10] Tamamoto T, Ohno K, Ohmi A, Goto-Koshino Y and Tsujimoto H. Verification of measurement of the feline serum amyloid A (SAA) concentration by human SAA turbidimetric immunoassay and its
clinical application. J VET MED SCI 2008, 70: 1247-1252.

[11] Kushner I. The acute phase response: an overview. Methods Enzymol 1988, 163: 373-383.

[12] Malle E and De Beer FC. Human serum amyloid A (SAA) protein: a prominent acute-phase reactant for clinical practice. EUR J CLIN INVEST 1996, 26: 427-435.

[13] Armstrong H, Bording-Jorgensen M, Dijk S and Wine E. The Complex Interplay between Chronic Inflammation, the Microbiome, and Cancer: Understanding Disease Progression and What We Can Do to Prevent It. Cancers (Basel) 2018, 10.

[14] Malle E, Sodin-Semrl S and Kovacevic A. Serum amyloid A: an acute-phase protein involved in tumour pathogenesis. CELL MOL LIFE SCI 2009, 66: 9-26.

[15] Biaoxue R, Hua L, Wenlong G and Shuanying Y. Increased serum amyloid A as potential diagnostic marker for lung cancer: a meta-analysis based on nine studies. BMC CANCER 2016, 16: 836.

[16] Yang M, Liu F, Higuchi K, Sawashita J, Fu X, Zhang L, Zhang L, Fu L, Tong Z and Higuchi K. Serum amyloid A expression in the breast cancer tissue is associated with poor prognosis. ONCOTARGET 2016, 7: 35843-35852.

[17] Ren Y, Wang H, Lu D, Xie X, Chen X, Peng J, Hu Q, Shi G and Liu S. Expression of serum amyloid A in uterine cervical cancer. DIAGN PATHOL 2014, 9: 16.

[18] Wood SL, Rogers M, Cairns DA, Paul A, Thompson D, Vasudev NS, Selby PJ and Banks RE. Association of serum amyloid A protein and peptide fragments with prognosis in renal cancer. Br J Cancer 2010, 103: 101-111.

[19] Chan DC, Chen CJ, Chu HC, Chang WK, Yu JC, Chen YJ, Wen LL, Huang SC, Ku CH, Liu YC and Chen JH. Evaluation of serum amyloid A as a biomarker for gastric cancer. ANN SURG ONCOL 2007, 14: 84-93.

[20] Cocco E, Bellone S, El-Sahwi K, Cargnelutti M, Buza N, Tavassoli FA, Schwartz PE, Rutherford TJ, Pecorelli S and Santin AD. Serum amyloid A: a novel biomarker for endometrial cancer. CANCER-AM CANCER SOC 2010, 116: 843-851.

[21] REN Yanjie, WANG He, LU Donghao, et al. Expression of serum amyloid A in uterine cervical
[22] Lu SY, Rodriguez M and Liao WS. YY1 represses rat serum amyloid A1 gene transcription and is antagonized by NF-kappa B during acute-phase response. MOL CELL BIOL 1994, 14: 6253-6263.

[23] Ren Y and Liao WS. Transcription factor AP-2 functions as a repressor that contributes to the liver-specific expression of serum amyloid A1 gene. J BIOL CHEM 2001, 276: 17770-17778.

[24] Nguyen KD, Macaubas C, Truong P, Wang N, Hou T, Yoon T and Mellins ED. Serum amyloid A induces mitogenic signals in regulatory T cells via monocyte activation. MOL IMMUNOL 2014, 59: 172-179.

[25] Wang JY, Zheng YZ, Yang J, Lin YH, Dai SQ, Zhang G, Liu WL. Elevated levels of serum amyloid A indicate poor prognosis in patients with esophageal squamous cell carcinoma. BMC Cancer. 2012; doi:10.1186/1471-2407-12.365.

[26] O'Hara R, Murphy EP, Whitehead AS, FitzGerald O and Bresnihan B. Local expression of the serum amyloid A and formyl peptide receptor-like 1 genes in synovial tissue is associated with matrix metalloproteinase production in patients with inflammatory arthritis. Arthritis Rheum 2004, 50: 1788-1799.

[27] Tamamoto T, Ohno K, Goto-Koshino Y and Tsujimoto H. Effects of serum amyloid A on matrix metalloproteinase-9 production in feline lymphoma-derived cell lines. Vet Immunol Immunopathol 2017, 187: 10-13.

[28] Deng G, Zhou F, Wu Z, Zhang F, Niu K, Kang Y, Liu X, Wang Q, Wang Y and Wang Q. Inhibition of cancer cell migration with CuS@ mSiO2-PEG nanoparticles by repressing MMP-2/MMP-9 expression. Int J Nanomedicine 2018, 13: 103-116.

[29] An J, Xue Y, Long M, Zhang G, Zhang J and Su H. Targeting CCR2 with its antagonist suppresses viability, motility and invasion by downregulating MMP-9 expression in non-small cell lung cancer cells. ONCOTARGET 2017, 8: 39230-39240.

[30] Zhao Y, Zhou S and Heng CK. Celecoxib inhibits serum amyloid A-induced matrix metalloproteinase-10 expression in human endothelial cells. J VASC RES 2009, 46: 64-72.

[31] Lee HY, Kim MK, Park KS, Bae YH, Yun J, Park JI, Kwak JY and Bae YS. Serum amyloid A stimulates
matrix-metalloproteinase-9 upregulation via formyl peptide receptor like-1-mediated signaling in human monocytic cells. Biochem Biophys Res Commun 2005, 330: 989-998.

[32] De Buck M, Gouwy M, Wang JM, Van Snick J, Opdenakker G, Struyf S and Van Damme J. Structure and Expression of Different Serum Amyloid A (SAA) Variants and their Concentration-Dependent Functions During Host Insults. CURR MED CHEM 2016, 23: 1725-1755.

[33] Urieli-Shoval S, Finci-Yeheskel Z, Dishon S, Galinsky D, Linke RP, Ariel I, Levin M, Ben-Shachar I and Prus D. Expression of serum amyloid a in human ovarian epithelial tumors: implication for a role in ovarian tumorigenesis. J HISTOCHEM CYTOCHEM 2010, 58: 1015-1023.

[34] Hanahan D and Weinberg RA. Hallmarks of Cancer: The Next Generation. CELL 2011, 144: 646-674.

[35] Tamamoto T, Ohno K, Goto-Koshino Y and Tsujimoto H. Serum amyloid A promotes invasion of feline mammary carcinoma cells. J VET MED SCI 2014, 76: 1183-1188.

[36] Shay G, Lynch CC and Fingleton B. Moving targets: Emerging roles for MMPs in cancer progression and metastasis. MATRIX BIOL 2015, 44-46: 200-206.

[37] Lee HY, Kim M, Park KS, Bae YH, Yun J, Park J, Kwak J and Bae Y. Serum amyloid A stimulates matrix-metalloproteinase-9 upregulation via formyl peptide receptor like-1-mediated signaling in human monocytic cells. BIOCHEM BIOPH RES CO 2005, 330: 989-998.

[38] Arvelo F, Sojo F and Cotte C. Tumour progression and metastasis. Ecancermedicalscience 2016, 10: 617.

[39] Ha GH, Park JS and Breuer EK. TACC3 promotes epithelial-mesenchymal transition (EMT) through the activation of PI3K/Akt and ERK signaling pathways. CANCER LETT 2013, 332: 63-73.

[40] Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES and Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005; 102:15545-15550.

[41] Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in
human diabetes. Nat Genet. 2003; 34:267-273.

[42]. Tazzari PL, Cappellini A, Grafone T, Mantovani I, Ricci F, Billi AM, Ottaviani E, Conte R, Martinelli G and Martelli AM. Detection of serine 473 phosphorylated Akt in acute myeloid leukaemia blasts by flow cytometry. Br J Haematol. 2004; 126:675-681.

[43]. Kuo YC, Huang KY, Yang CH, Yang YS, Lee WY and Chiang CW. Regulation of phosphorylation of Thr-308 of Akt, cell proliferation, and survival by the B55alpha regulatory subunit targeting of the protein phosphatase 2A holoenzyme to Akt. J Biol Chem. 2008; 283:1882-1892.

[44] Coloff JL, Mason EF, Altman BJ, Gerriets VA, Liu T, Nichols AN, Zhao Y, Wofford JA, Jacobs SR, Ilkayeva O, Garrison SP, Zambetti GP and Rathmell JC. Akt requires glucose metabolism to suppress puma expression and prevent apoptosis of leukemic T cells. J Biol Chem. 2011; 286:5921-5933.

Tables

Table 1 Clinicopathological features of patient samples

| Characteristics          | Number of cases(%) |
|--------------------------|--------------------|
| Age(y)                   |                    |
| ≥60                      | 55(55)             |
| <60                      | 45(45)             |
| FIGO stage               |                    |
| I-II                     | 30(30)             |
| III                      | 25(25)             |
| IV                       | 45(45)             |
| Pathologic type          |                    |
| Serous                   | 64(64)             |
| Mucinous                 | 36(36)             |
| Lymphatic invasion       |                    |
| No                       | 41(41)             |
| Yes                      | 59(59)             |
| Distant metastasis       |                    |
| NO                       | 55(55)             |
| Yes                      | 45(45)             |
| ER                       |                    |
| Positive                 | 70(70)             |
| Negative                 | 30(30)             |
| PR                       |                    |
| Positive                 | 39(39)             |
| Negative                 | 61(61)             |

Table 2 Univariate analysis of the association of various clinicopathological features with SAA expression of ovarian cancer
Table 3: Multivariate Cox regression analysis of the association of various clinicopathological features with SAA expression of ovarian cancer in entire cohort

| Feature                      | B   | S.E  | Wald | df | Sig.  | Exp(B) | 95% CI          |
|------------------------------|-----|------|------|----|-------|--------|-----------------|
| FIGO stage                   |     |      |      |    |       |        | Lower          |
| I-II                         | 1.156 | 0.598 | 6.813 | 1 | 0.009 | 4.758 | 1.47 \(5\) 15.350 | Upper          |
| III                          | 2.457 | 0.871 | 7.955 | 1 | 0.004 | 11.667 | 2.11 \(6\) 64.326 |                |
| IV                           | 2.799 | 1.008 | 7.715 | 1 | 0.005 | 16.430 | 2.27 \(9\) 118.424 |                |
| Lymphatic invasion           | 2.890 | 1.219 | 5.622 | 1 | 0.018 | 18.000 | 1.65 \(0\) 196.309 |                |
| Distant metastasis           | 3.632 | 1.166 | 9.710 | 1 | 0.002 | 37.800 | 3.84 \(9\) 371.271 |                |

Table 4: The median serum SAA, CA125, and HE4 concentration

| Group              | N    | SAA [mg/L, M(QR)] | CA125 [U/L, M(QR)] | HE4 [pmol/L, M(QR)] |
|--------------------|------|------------------|-------------------|---------------------|
| Health             | 30   | 4.95 (3.27-7.67) | 17.79 (10.48-30.77) | 21.39 (14.38-30.71) |
| Benign disease     | 36   | 6.10 (4.70-9.45) | 35.86 (15.82-53.16) | 47.73 (37.39-74.64) |
| Ovarian cancer     | 36   | 32.30 (11.20-119.6) | 640.00 (251.65-1218.50) | 259.5 (125.00-736.00) |

Table 5: Area under the curve and cut-off value

|                     | cut-off | AUC   | YOUDE | Sensitivity | Specificity | 95% CI |
|---------------------|---------|-------|-------|-------------|-------------|--------|
| SAA (mg/L)          | 17.05   | 0.889 | 0.649 | 0.784       | 0.865       | 0.861  |
| CA125 (U/L)         | 245.30  | 0.868 | 0.730 | 0.811       | 0.919       | 0.778  |
| HE4 (pmol/L)        | 98.01   | 0.917 | 0.703 | 0.865       | 0.838       | 0.857  |
| Combined            | 0.945   | 0.838 | 0.892 | 0.973       | 0.891       | 0.999  |

Figures
SAA is overexpressed in ovarian cancer. A. SAA is overexpression in ovarian cancer samples (109 cases) compared with adjacent normal tissue samples (37 cases) in Oncomine profiles. B. SAA expression was markedly increased in 37 paired ovarian tumor tissues (Tumor) and their adjunct normal tissues (Adjacent) in Oncomine profiles. C. Kaplan-Meier survival curves demonstrated that the overall survival of patients with high expression of SAA was significantly shorter than those with low SAA expression in the same profiles.
Figure 2

SAA as a prognostic biomarker in ovarian cancer. A. Heat-map of SAA between pre-treatment (A) and post-treatment (B). n=20, ** P<0.01. B. Comparison of SAA expression in serum among healthy control group, ovary (benign and cancer) group. C. SAA expression in serum among healthy control group, ovarian cancer group for the patients with stage I/II and the patients with stage III/IV. **** P < 0.001. *** P<0.001,* P<0.05. D. ROC curve of sensitivity versus specificity of SAA, CA125, HE4 and combinations of three makers.
SAA modulates migration of ovarian cancer cells. A. Expression of SAA-1, SAA-2 and SAA-4 in ovarian tumor tissues and OVCAR3 cell. B. cDNA from 5 ovarian cancer tissues and paracancerous tissues were analyzed for SAA-1 and SAA-4 levels by qRT-PCR. (C,D). The data are shown as the means ±SD, ****, P<0.0001, *, P<0.05. Kaplan-Meier overall survival analysis of SAA-1 (C) and SAA-4 (D) expression in ovarian cancer from Human Protein Atlas available (www.proteinatlas.org). Expression cutoffs were set to 9.8 FPKM (C), 0.0 FPKM (D).
AKT signaling pathway is regulated by SAA. A. OVCAR-3 cell was treated with SAA 0μg/ml and 10μg/ml 12h respectively, representative graphs of OVCAR-3 cells in migration. The data were shown as the means±SD (n = 3). B. The mRNA of MMP-1, MMP-9 and MMP-12 were
assayed after OVCAR-3 cell been treated with SAA 0μg/ml and 10μg/ml 24h respectively. The data were shown as the means±SD (n = 3). (C,D) RT-qPCR and Western blot analysis of EMT markers in OVCAR-3 cell treated with SAA 0μg/ml and 10μg/ml 24h respectively. The data were shown as the means±SD (n = 3). E.Western blot analysis of phosphorylation Akt in OVCAR-3 cell treated with SAA 0μg/ml and 10μg/ml 24h.